1-1-1987

Indirect photometric chromatography of iodide ion in aqueous solutions

M. Liz Allyn

INDIRECT PHOTOMETRIC CHROMATOGRAPHY OF IODIDE ION IN AQUEOUS SOLUTIONS

M. LIZ ALLYN

JANUARY 1987

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

APPROVED:

Dr. Paul Rosenberg
Project Advisor

Department Head

Library

Rochester Institute of Technology
Rochester, New York 14623
Department of Chemistry

Title of Thesis Indirect Photometric Chromatography of Iodide Ion in Aqueous Solutions

I M. Liz Allyn hereby grant permission to the Wallace Memorial Library, of R.I.T., to reproduce my thesis in whole or in part. Any reproduction will not be for commercial use or profit.

Date January 15, 1987

To Mom and Dad

ACKNOWLEDGEMENTS

I wish to acknowledge the help of Dr. Paul Rosenberg and thank him for the direction and encouragement necessary to complete my degree. I also want to thank Dr. Kay Turner, Dr. Laura Tubbs, Dr. Terence Morrill, Dr. Jerry Adduci, and Dr. Hornak for being on my oral committees. Finally, I wish to give thanks for the support of my parents and family who greatly helped in realizing my goal.

TABLE OF CONTENTS

Abstract 1

Introduction 2

Table I Methods of Separation 4
Table II Ion Exchange Reactions 7
Figure 1 Schematic of Ion Exchange 9
Figure 2 Instrumentation Used in IC 14
Figure 3 Hollow Fiber Ion Exchange Suppressor. 17
Figure 4 An Example of IPC 22
Figure 5 Separation of the Halides by IPC 24
Figure 6 Instrumentation Used in IPC 26

Experimental 30

Results and Discussion 32

Figure 7 Examples of IPC Chromatograms 36
Table III Standardization of $Cu(NO_3)_2$ Eluent 37
Figure 8 Peak Elution as a Function of pH 39
Table IV Comparison of I^- Elution Volumes 40
Figure 9 Effect of Eluent pH on Selectivity 41
Figure 10 Distribution of Trimesate Species 46
Figure 11 Chromatograms of the Varian 5060 LC 48
Figure 12 Distribution of Phthalate Species 53
Figure 13 Irreversible Column Damage 55
Table V Optimum Separation Conditions 57

References 59

ABSTRACT

Indirect photometric chromatography was applied to the detection and quantification of iodide ion in aqueous solutions. A Nucleosil SB column containing the trimethylamine functional group was utilized in this HPLC study to compare the selectivity and reproducibility of copper (II) nitrate, trimesic acid, and phthalic acid eluents. Trimesic acid eluent was the strongest eluent, $R_t(I^-)$=3.5min, followed by phthalic acid, $R_t(I^-)$=14.6min, and copper nitrate, $R_t(I^-)$=15.5min, assuming optimum conditions. Both aromatic eluents were held onto the column by adsorption reactions with the styrene-divinylbenzene matrix of the resin. Irreversible column damage was the result. Coupled with the fact that the copper nitrate was such a weak eluent, it was concluded that these eluents were unsuitable for the IPC method in obtaining quantitative results in the detection of iodide ion.

INTRODUCTION

The overall goal of this study is to develop a method to detect and quantify iodide ion in aqueous solutions. This introduction references standard methods of determining iodide ion and compares the results obtained with those expected for the new method. High performance liquid chromatography (HPLC) and the ion exchange mode of separation are discussed as are the necessary mobile and stationary phases used to carry out the separation of sample components. The instrumentation and typical output for the ion chromatographic method is illustrated.

Previous methods determining iodide ion are primarily wet chemical methods such as gravimetric precipitations, potentiometric titrations and volumetric titrations (1). Gravimetric analyses (2) precipitate AgI or PdI_2 to determine iodide ion. Other halides, sulfide, cyanide, and other anions may interfere by coprecipitating with the silver or palladium cations. This method is time consuming with many possible errors so other procedures are generally preferred.

Platinum and calomel electrodes in a dilute solution of sulfuric acid can be used to determine iodide ion. A potentiometric redox titration is performed with permanganate ion as the titrant which reduces the iodide ion in the presence of sulfuric acid. Interferences are small and halides may be present in the solution to be analyzed. The potential across the electrodes is monitored and the endpoint is reached at +1.0 volt versus the standard calomel

electrode (S.C.E.). Switching the supporting electrolyte to nitric acid and the titrant to silver ion produces a potentiometric precipitation titration. Silver iodide precipitates at the endpoint of +0.102 volt against the S.C.E. The details of similar conductometric, amperometric, complexometric, and volumetric redox titrations are not included here, but may be performed with a variety of reagents. Results have a precision of $\pm$0.2-1% and an accuracy of 0.02% if the optimum conditions are achieved.

A prime problem of the above methods is that they are difficult to perform on trace amounts. The development of a new method would have to be efficient and easy to perform. Results obtained must have excellent precision and accuracy or the method would not merit employment over the older, wet chemical methods. In satisfying these goals HPLC appears to be most beneficial as an analytical technique. HPLC is rapid and easy to perform; typical determinations take 3-10 minutes. The sample size may vary from 5 µL to 5 mL depending on the application, and quantitative results are obtained reproducibly into the part per million range.

HPLC is a form of liquid-solid chromatography which employs a liquid as the mobile phase and some type of solid support as the stationary phase. A wide range of concentrations and a combination of solvents can be utilized as the mobile phase to enhance the selectivity of the method. The stationary phase can also be a variety of separating materials which can further enhance the method's selectivity. The proper choice of mobile and stationary phases is determined by the chemical nature of the solutes to be detected. In this study the goal is detection and quantification of a known solute, I^-. Iodide ion is water soluble

and ionic so the most effective mode of separating iodide ion from other solutes in aqueous solutions is ion exchange (3). Before looking at the chromatography of ions however, one must first understand the mechanism of the separation.

Table I shows the four modes of separation employed in chromatography (4). These methods of separation should not be confused with laboratory operations. Adsorption can be carried out experimentally in a column, on paper or on a thin layer. The basic principle of separation, adsorption, is the same for each operation. The chromatography of ions focuses interest on the ion exchange mode of separation and relies on the reversible exchange of ions as applied in HPLC operations.

Table I

Methods of Separation

Mode	Basis of Separation
adsorption	intermolecular forces
partition	intramolecular interactions
exclusion	molecular size
ion exchange	reversible exchange of ions

The concept of ion exchange is illustrated if water is allowed to percolate through layers of sands or clay minerals. The sands and minerals exchange ions with the water in a naturally occurring purification process. Upon analysis it is noted that certain ions are removed from the water, particularly potassium and ammonium ions. An equivalent amount of another cationic species is released into the water, such as calcium or sodium ions. By taking advantage of this purification process calcium ions can be removed from hard water. Water softening agents are typically from a class of silica-

containing minerals having empirical chemical formulas such as $Na_2Al_2Si_4O_{12}$. These minerals are called zeolites and exist in equilibrium:

$$Ca^{2+} + Na_2Al_2Si_4O_{12} \rightleftharpoons 2Na^{+} + CaAl_2Si_4O_{12}$$

Calcium ions in the hard water will be removed until equilibrium is achieved and there is a balance of free calcium and sodium ions. That does not mean there will be an equal concentration of these free ions in solution due to ion exchange. Rather, the rate of ion exchange and the degree to which equilibrium occurs is a function of the free ion concentration and the relative affinities of each ion for the zeolite.

In the preceding example the calcium ions are shown to have a greater affinity for the ion exchange material than the sodium ions because the calcium ions are ultimately bound to the zeolite. There are several factors which determine relative affinities, including the state of charge of the ion being exchanged. Calcium (II) is in a higher state of charge than sodium (I). In a solution containing approximately equal concentrations of these two species the calcium ions would have a higher affinity for the ion exchange material. Due to its greater charge the calcium ion will bind more tightly to the zeolite.

Applying Le Chatelier's Principle the zeolite can be regenerated by subjecting it to a flow of an excess of sodium ions. The equilibrium is disturbed and the reaction is forced to the left. Calcium ions, which had previously been removed from the water, are

now released into solution and the ion exchange material is regenerated into its sodium form.

The zeolite materials contain ionic sites which strongly interact with ionic solutes in the water. These ionic sites are inherent to the composition of these inorganic minerals which make up the solid support or the stationary phase of the chromatographic system. Temporary ionic bonds are created when an ion of opposite charge from the solution is in the proximity of an ionic site on the resin. The newly created bonds are broken as other solutes pumped through the system have greater attractions for the ionic sites and bind there by creating new ionic bonds. The solute previously held at the charged site is released into the mobile phase and is free to travel to another charged site on the stationary phase.

Separation occurs as each solute binds the stationary phase for longer or shorter periods of time according to the degree of attraction each has for the ionic sites on the stationary phase. Those with a greater attraction are held for relatively long periods of time and elute from the chromatographic column later than the ions with a smaller attraction for the ionic sites.

Recently, many synthetic organic ion exchange resins have been developed which are superior to the inorganic exchange materials. Organic resins exhibit chemical stability, mechanical strength, exchange capacity and a rate of ion exchange greater than their inorganic counterparts (5).

There are two types of ion exchangers. One type of ion exchanger has a negatively charged site, or functional group, which exchanges cations. A second type exchanges anions at the positively charged site on the resin. Examples of each are shown below:

Table II

Ion Exchange Reactions

Cation Exchange

$$\text{Resin-SO}_3^-\text{H}^+ + \text{MX} \rightleftharpoons \text{Resin-SO}_3^-\text{M}^+ + \text{HX}$$

Anion Exchange

$$\text{Resin-NR}_3^+\text{OH}^- + \text{HX} \rightleftharpoons \text{Resin-NR}_3^+\text{X}^- + \text{H}_2\text{O}$$

Each functional group is written as a closely associated ion pair to illustrate how ion exchange occurs. The aqueous forms of the sulfonic acid and ammonium groups are shown to be the active sites on the resin backbone for cation and anion exchange, respectively. In cation exchange the sulfonic acid group gives up a proton for a sodium ion, i.e. a hydrogen ion is exchanged for a sodium ion, or any other cation. An exchange reaction also occurs during anion exchange as the ammonium group gives up an hydroxide ion for another anion. The resin molecule is usually a polystyrene polymer in the form of spherically shaped cross-linked beads. The beads are treated to give a functionality producing either cation or anion exchange reactions.

Ion exchange as a mechanism of separation relies on the fact that the functional groups on the resin and the sample species are ionic. The anion exchange resin contains trialkylamine groups that are permanently charged in aqueous solutions. The charge is balanced by a counter ion from the eluent. As the eluent is pumped through the column a sample anion replaces the eluent anion which is

bound to the functional site and then an eluent anion will replace the sample anion and so on. Each step is referred to as a theoretical plate and is the length of the column required for an ion to be exchanged from mobile phase to stationary phase and back to the mobile phase. Figure 1 illustrates this process and the temporary bonds made and broken again as the eluent and sample species are pumped through the system. In this example the eluent species are the hydroxide anions and the sample species are the fluoride and chloride anions.

On a molecular level the selectivity of an ion exchange process exhibits the properties of the ions being exchanged. The ion exchange resin strongly retains ions of higher charge, ions of smaller solvated equivalent volume, and ions of greater polarizability (6). Each of these three types of properties will cause ions to be retained for longer periods of time on the column due to enhanced interactions with the resin or with the functional sites on the resin. Smaller ions will be strongly retained in the pores of the resin while ions of higher charge and greater polarizability will more tightly bind to the functional sites on the resin. The total retention time of an ion pumped through a chromatographic system is due to a combination of these interactions. All three types of interactions may occur; however, one type usually is stronger than the other for a given ion.

The electrostatic field, or the area of charge, around a highly charged ion is greater than the electrostatic field of an ion of comparable size having a smaller charge. The large electrostatic field will bind more tightly to a site of opposite charge, such

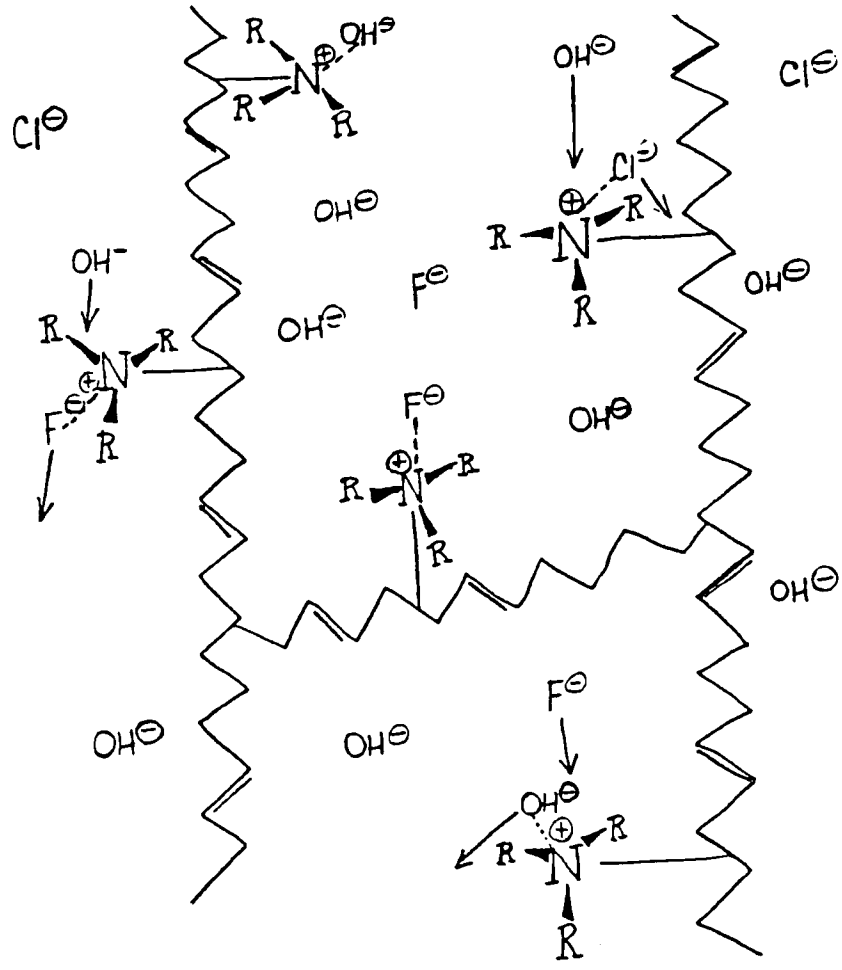

FIGURE 1--Schematic of Ion Exchange

The zig-zag lines in this drawing represent the resin backbone containing some cross-linking. The segments do not represent a carbon-to-carbon backbone in the classical sense, but rather the three dimensional structure of the resin. The trialkylamine groups, $-NR_3^+$, which are charged in the aqueous solution, show bonding to the anions in solution. Hydroxide anions are eluent species, the fluoride and chloride anions are sample species, and the R substituents of the functional groups are commonly methyl or methyl and hydroxyethyl groups. The arrows illustrate exchange of anions taking place as a new anion is attracted to the functional group and the current counter ion is displaced into solution.

as the functional group on the exchange resin. Exchanging either cations or anions, the functional group retains ions of higher charge for longer periods of time than ions with smaller charges. This can be illustrated by thinking of an ion exchange column as having a certain number of functional groups capable of exchanging ions, i.e., as having a defined length. Ions of different states of charge start at the top of the column at the same time. As each ion travels through the column each is bound by the functional group on the resin for a certain length of time. Stronger interactions bind the ions having a higher charge for longer periods of time due to the larger electrostatic field of these ions. Ions of smaller charge have relatively weak interactions with the functional groups and are subsequently passed through the column more quickly than ions of higher charge. Each ion interacts with the same number of functional groups, but is retained on the column for different amounts of time because each has a different electrostatic field.

The resin is a hydrophobic, hydrocarbon polymer. In aqueous solutions the cross-linked beads of the polymer swell due to the water molecules entering the beads and solvating the functional groups. Those ions of a smaller solvated equivalent volume will be better accomodated within the bead. Ions taking up less space will spend more time inside the polymer, in the pores of the bead.

Ions of greater polarizability will bind to the charged site on the resin longer. The polarizability of an ion refers to the ease with which charges in that ion can be directed. Redirecting the charges in an ion by placing another charge in the vicinity of that ion deforms the electron cloud and induces a dipole in the ion which is attracted to the nearby charged site on the resin. This results

in increased binding to the resin. Strongly polarizable ions can have well defined areas of charge which allow for very strong binding to the ionic site or functional group. Examples of ions that are polarizable are large ions having their outer electrons far from the nucleus. The iodide ion is an example of a highly polarizable ion. It is the largest common ion in the halide series and has the electron configuration [Kr] $5s^2 4d^{10} 5p^6$. Containing a filled p-orbital, the iodide ion is highly polarizable and usually has a relatively long retention time compared to ions of low polarizability.

Ion exchange has been applied to a variety of organic and inorganic ions so it is understandable that a variety of separating resins have been developed. Cation and anion separators are typically prepared from starting materials of styrene and divinylbenzene (7). Styrene (S) and divinylbenzene (DVB) are catalytically polymerized to give a network of interlaced polystyrene chains with divinylbenzene cross-linking units. The degree of cross-linking is controlled by the amount of divinylbenzene added in the production of the copolymer. In commercially produced resins the amount of divinylbenzene varies from 2 to 12% depending on the desired properties of the end product (8). The resins with greater cross-linking allow only small ions and molecules to penetrate the matrix. At this point in the manufacturing process the styrene-divinylbenzene copolymer is in the shape of spherical beads. The beads are further treated in a sulfonation reaction to introduce the active sulfonic acid groups.

Conditions of the sulfonation reaction can be varied to obtain the functional sulfonic acid groups just on the surface of the beads. This is done by heating a quantity of 2% DVB resin in 100°C sulfuric acid for a few minutes. The result is a thin shell of sulfonic acid groups on the surface of the resin. The surface-sulfonated-styrene-divinylbenzene (SSS/DVB) cation exchange resin is noted for its very low exchange capacity of 0.02 meq/gram copolymer (9). The capacity of a resin is the amount of functional groups per mass unit of exchange resin that is available for exchange (10).

A method was developed to convert the SSS/DVB cation exchanger to an anion exchanger (11) by first grinding a strong anion exchanger, like Dowex 1, collecting the fine particles, on the order of 0.5-2.0 μm, by removal of the larger particles by sedimentation. A dilute suspension of the fine particles is then passed through a column packed with the SSS/DVB cation exchange resin. The negatively charged fine particles become agglomerated to the surface of the resin at the positively charged functional group. The polycationic and the polyanionic materials have a strong electrostatic interaction which allows the two materials to "clump" together. When the column is saturated the excess fines elute from the column and the column is rinsed with water and ready to use as an anion exchanger.

Small, Stevens and Bauman (12) applied the ion exchange mode of separation in HPLC to the detection of inorganic and organic ions in solution using these ion exchange resins. This technique was termed ion chromatography, IC. The original ion chromatographs contain two ion exchange columns arranged in series which are

coupled to a conductivity detector. As shown in Figure 2 the remaining instrumentation includes solvent reservoirs containing the mobile phase or eluent, a high pressure pumping system, an injection valve, a chart recorder and a waste collector.

An example separation is presented. One solvent reservoir contains sodium carbonate as the eluent or mobile phase. A sample of fluoride and chloride ions, in a solution of NaF and NaCl, is introduced or loaded into the injector at the sample inlet of the injection valve. Turning the injector knob sends the sample to the head of the first column, the separator, which contains an anion exchanger in the carbonate form, $R_2^+CO_3^{2-}$. Separation is based on the selectivity, or affinity, that each anion has for the ion exchange resin in competition with the CO_3^{2-} from the eluent (13). Those anions having a strong affinity for the exchange resin stay on the column longer than other species which do not strongly interact with the exchange resin. Reversible exchange of ions occurs to selectively retain each anionic species to a certain extent. The fluoride and chloride ions are separated at this point and exist primarily as F^- and Cl^- in aqueous solution. The sodium counter ions are not retained and pass through the system. Conductometric detection at this point would be impractical due to the large background interference of the ionic species, Na^+ and CO_3^{2-}, from the sodium carbonate eluent (14).

To overcome this detection problem a strong acid cation exchanger in the hydrogen form, R^-H^+, is used in the second column, the suppressor. The purpose of the suppressor column is to exchange ions of opposite charge that may interfere with electro-

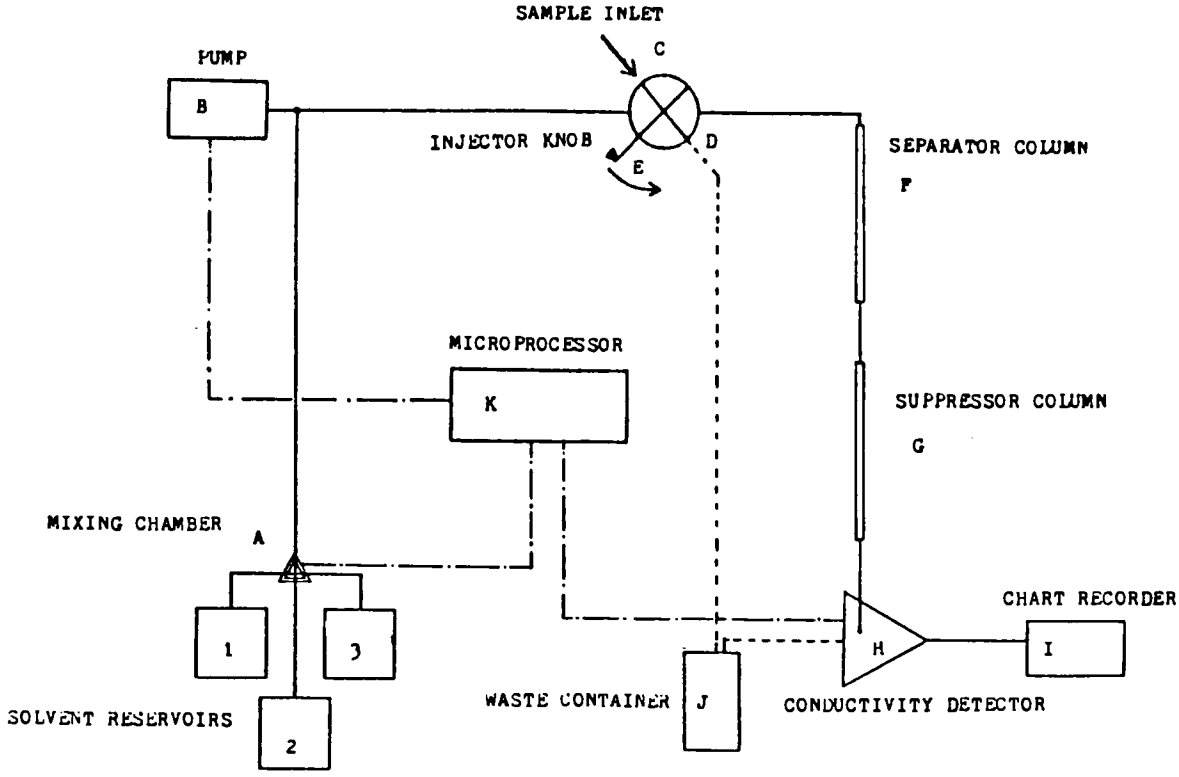

FIGURE 2--Instrumentation Utilized in Ion Chromatography

Solvents contained in reservoirs 1, 2, or 3, or some combination of the three, are drawn into the mixing chamber, A, and thoroughly mixed. The microprocessor, K, is used to control the eluent composition and flow rate by adjusting the pump, B, and the inlet valves for the mixing chamber, A. The sample is introduced at C, which is the sample-in port of the injector. When the sample loop is full, any excess sample is sent via point D to a waste container, J. Turning the injector knob, E, by 45 degrees allows the eluent to be pumped through the sample loop. The sample is pumped to the head of the first column, the separator column, F, which separates the sample into its ionic components by ion exchange. The sample components are pumped into the suppressor column, G, which reduces the background conductivity from the eluent. The suppressor column is necessary to allow better detection of the sample components in the conductivity detector, H, which is also microprocessor controlled. The changes in conductivity of the eluent are recorded by the chart recorder, I. The used eluent and sample are discarded into the waste container, J.

chemical detection of the species of interest. Sodium cations are reversibly exchanged for hydrogen ions as the eluent is pumped through the suppressor column. The equilibrium responsible is the following:

$$R^{-}H^{+} + Na^{+}_{(aq)} + CO_3^{2-}{}_{(aq)} \rightleftharpoons R^{-}Na^{+} + H^{+}_{(aq)} + CO_3^{2-}{}_{(aq)}$$

where $R^{-}H^{+}$ represents the resin cation exchanger and (aq) shows the free species in solution. The sodium carbonate eluent is in its acidic form as it leaves the suppressor column:

$$2H^{+} + CO_3^{2-} \rightleftharpoons H_2CO_{3(aq)} \; ; \; K=4.8 \times 10^{16}.$$

As a result this effluent is much less ionized and therefore less conducting than the eluent entering the suppressor column. Because the eluent conductivity is reduced before the detection of sample species occurs, the sensitivity of the method is greatly increased.

A problem with this method is suppressor exhaustion. After several hours of use the active sites on the cation exchange resin, $R^{-}H^{+}$, are changed to inactive sites, $R^{-}Na^{+}$. As the eluent flows through the suppressor column, the suppressor becomes saturated with sodium ions and is no longer effective in suppressing the conductivity of the eluent. The ion exchange resin in the suppressor is depleted making it necessary to change or replenish the column which ultimately results in instrument down time.

Changing the suppressor column back into its active form is called regenerating the column. In this example the inactive sites, $R^{-}Na^{+}$, must be regenerated to active sites, $R^{-}H^{+}$, to continue

suppressing the eluent conductivity. According to Le Chatelier's Principle the equilibrium will be forced to the left if something is added on the right. This is accomplished by pumping a weakly acidic solution, 1×10^{-3} molar HCl, through the column for a period of time. Due to the greater concentration of hydrogen ions, regeneration occurs as sodium ions are exchanged for hydrogen ions. The functional groups of the resin are converted back into the original form, $R^{-}H^{+}$, and the effluent contains Na^{+} and CO_3^{2-}.

Automated reconditioning steps have been implemented in an attempt to overcome the problem of suppressor exhaustion. One example is to make the suppressor a hollow fiber ion exchange membrane (15) as shown in Figure 3. A steady flow of hydrogen ions in the form of dilute sulfuric acid is passed over the outside of the membrane allowing hydrogen ions, H^{+}, to exchange through the membrane for sodium ions, Na^{+}. This exchange of ions reconditions the eluent and permits continuous operation of the IC system.

Further development of the method has lead to packing the hollow fiber suppressor with inert beads, which reduces the band spreading and gives better resolution (16). The use of a nylon monofilament cation exchanger membrane in an annular helical configuration has been demonstrated to allow highly efficient mass transfer to the walls of a tube (17). The most rapid regeneration of the eluent is performed using this configuration because more species are brought into contact with the regenerator acid in a given amount of time. This type of suppressor is described as one of low dead volume and as one which introduces only a small amount of peak dispersion as band broadening (18).

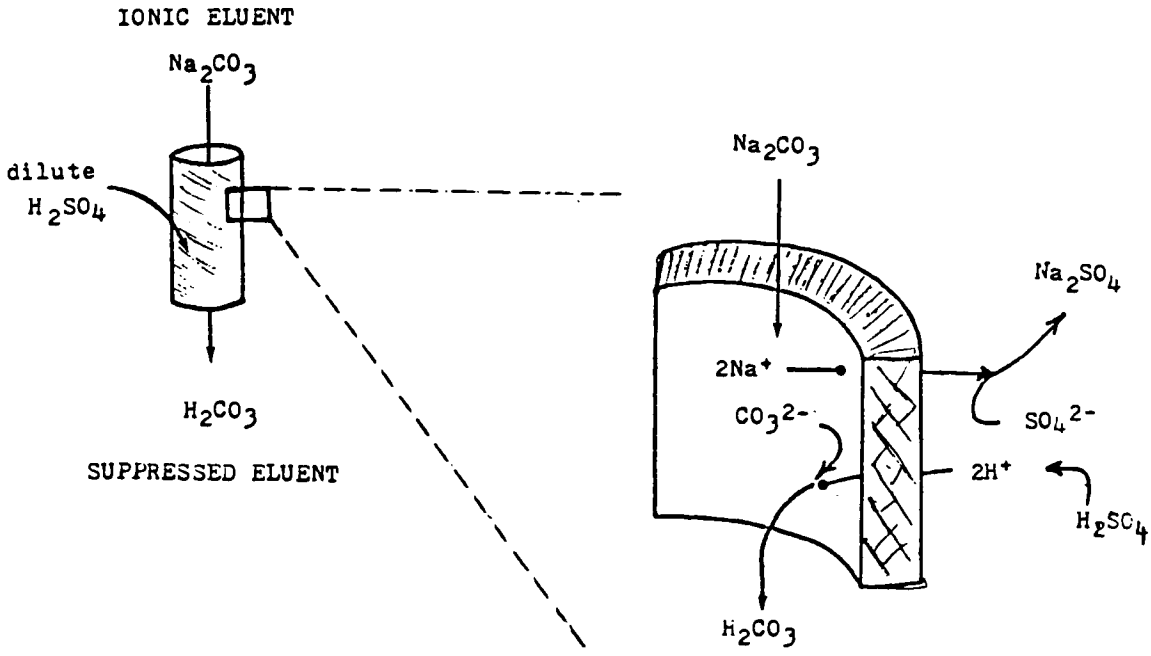

FIGURE 3--Hollow Fiber Ion Exchange Suppressor

This type of suppressor column is a cation exchange membrane in an open tubular form. The sample components and the eluent pass through the center of the hollow fiber while at the same time a counter flow of dilute acid is passed over the outside of the fiber. Cations in the eluent stream, Na^+, are exchanged through the membrane for hydrogen ions, H^+, while obeying the laws of electroneutrality. This allows each ionic species entering the top of the suppressor column to be converted to its acid form as it contacts the wall of the suppressor fiber. The result is decreased ionicity and conductivity of the eluent and better detection of the sample components.

Another method of preventing suppressor exhaustion is to couple a low capacity separator column with a high specific capacity suppressor column (19). Solutes travel relatively quickly through a low capacity column because there are fewer active sites on the resin with which to exchange. Therefore, the eluent concentration may be diluted to give optimum separation of components in the low capacity column without sacrificing separation efficiency. Only a small portion of the high capacity suppressor column would be used with the dilute eluent and thus prolong the suppressor column life.

Even if regeneration of the suppressor column is not a problem, the suppressor column and its related hardware increase the void volume of the system. This leads to higher detection limits and poorer resolution due to band spreading. Band spreading or zone broadening is produced in any chromatographic system. The band or zone that is enlarged is the area that the sample or solute molecules occupy. Three mechanisms account for the observed change in the chromatographic zone: diffusion, slow equilibration, and flow patterns (20).

Diffusion is the result of the natural movement of the molecules in the mobile phase. Starting out as a thin zone at the top of the column, the molecules spread out over a period of time into a Gaussian profile.

Slow equilibration of the chromatographic process is manifested in the kinetics of the solute molecule transfer from mobile phase to stationary phase. Solute molecules can be moved around faster in the mobile phase than in the stationary phase, so the chromatographic zone in the mobile phase is always ahead of the zone in the stationary phase. The overall zone is considerably broadened.

Flow patterns throughout the column are numerous. Each molecule may take a different path in reaching the end of the column. The zone is broadened into a Gaussian profile here also, as some molecules may travel a bit longer path than the average and some others may travel for a shorter path in a given length of time.

Each of these mechanisms occurs to some extent in broadening the solute zone inside the column of a chromatographic system. Little can be done to change these inner column problems because they are inherent in the techniques used to pack the stationary phase in the column and of the stationary phase itself. However, outside the column two more factors result in a broader zone than desired. The first is that each sample introduced into the column must occupy a finite space which is called the feed volume. Most often the sample is mixed with the eluent and then sent to the head of the column, so the sample is diluted into a broader zone at the start of the chromatographic run.

Mixing chambers are inherent to the design of the chromatograph and make up the second outside column effect of zone spreading. A mixing chamber is some area where the zone may enter and mix with the mobile phase, thus diluting the zone. Examples of mixing chambers would be lengths of tubing before and after the column, the detector flow cell, and the entire suppressor-regenerator system. Changes to the system in minimizing band spreading must be made to obtain better resolution. Using small sample inlet volumes and eliminating extra long tubing are two ways to accomplish this. Removal of the suppressor system and its related hardware would also help to increase resolution.

Other problems of the basic IC system include the fact that it is a dedicated system. Analyses other than those of ionic solutions are not applicable so the versatility of the IC system is limited. A basic ion chromatograph of this type costs from $10,000 to $17,000 (21) so the instrumentation can be expensive for those on a limited budget.

Many of the undesirable or unwanted characteristics of ion chromatography stem from the use of the suppressor column in conditioning the eluent for conductometric detection. Variations on the method are basically suppressorless techniques and most methods apply different modes of detection. Conductometric detection without suppression is referred to as non-suppressed ion chromatography and can take place providing that a significant difference exists in conductivity of the eluent and sample species (22,23). A powerfully displacing, although dilute eluent must be used to produce a weakly conducting background. Few choices of eluents limit the usefulness of this method. For cation analysis mineral acids are good eluent choices; for anion analysis aromatic acid salts are employed as eluents.

By using photometric detection the problems of limited eluent choice, poor signal detection due to interferring background of eluent, and extra hardware do not arise. Common UV detectors can now extend down to 200 nm or less allowing monitoring of the eluent at wavelengths not previously accessible. Low capacity exchangers are also used in this modified ion chromatography method (24,25).

Another version of ion chromatography has been developed which uses photometric detection, but in an indirect manner. Species transparent in the UV-VIS region, as are many inorganic ions, may

be detected by this mode. But how can a photometer be used to detect transparent ions in solution?

Indirect photometric chromatography (IPC) (26) monitors a decrease in the absorbance of the mobile phase when a non-absorbing solute elutes. An example may best explain the methodology. Suppose that an anion exchange column is equilibrated with an electrolytic solution, represented as Na^+E^-. All the exchange sites on the anion exchange column are saturated with eluent anions, E^-, and the concentration of each specie is monitored by an absorbance detector. Steady levels of Na^+ and E^- are obtained as long as the solution of these anions is allowed to flow uninterrupted. The unique feature of this system is that the absorbance of E^- is greater than 1.0 absorbance unit, 1.0 a.u. If concentration was monitored as a function of absorbance, output may be represented as in Plate #1 of Figure 4.

When an inorganic sample is injected the balance of the system is interrupted. Sample anions, S^-, are taken up by the anion exchange sites of the stationary phase and are retained for a certain length of time. As these species later elute from the column, the change in concentration must be recorded to effectively monitor the separation of the sample components. The absorbance detector can not directly monitor this change because the inorganic sample anions, S^-, do not absorb light in the range of the detector. However, by monitoring the absorbance of E^- at a fixed wavelength, the change in sample ion concentration, $[S^-]$, can be indirectly recorded. Since the concentration of Na^+ is constant throughout the system it follows that the total anion concentration remains fixed.

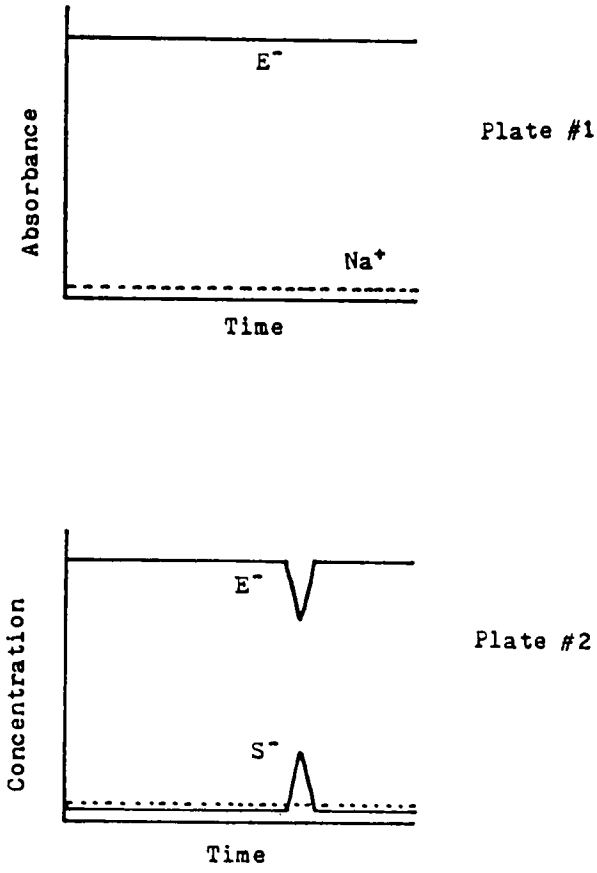

FIGURE 4--An Example of Indirect Photometric Chromatography

The unique feature of the IPC method is shown in plate #1 where the eluent anion, E^-, is a highly absorbing specie. The anion concentration is constant with time and is monitored with an absorbance detector because absorbance is proportional to concentration. After sample introduction and subsequent elution from the column, the change in eluent concentration is recorded as in plate #2. When the sample elutes from the column its concentration increases and the eluent anion concentration decreases, thus obeying the laws of electroneutrality. By monitoring the eluent absorbance the change in sample ion concentration is indirectly recorded as a characteristic trough.

Plate #2 of Figure 4 illustrates the relative change in anion concentration and also shows how the laws of electroneutrality are obeyed. The anionic content of the mobile phase is a constant throughout the system. When the concentration of sample anions exiting the system increases, i.e., when a sample component elutes from the column, there is a concomitant decrease in the eluent anion concentration. Therefore, $[S^-]$ can be indirectly recorded by continuously monitoring the absorbance of E^-. By monitoring a highly absorbing eluent as the baseline, the recorded "troughs" in the baseline will occur as a function of sample ion concentration.

Figure 5 is an example of a chromatogram obtained utilizing indirect photometric detection. The sodium phthalate eluent was monitored at 275 nm and shows the complete separation of the halides. Identity of each peak in Figure 5 was obtained by running standard solutions (27). Note the characteristic negative deflections for each ion.

The first peak or positive deflection is called the "pseudo" peak and it represents the total salt concentration injected (28). It is called the "pseudo" peak because it does not represent one particular sample species but a mixture of eluent anions and sample cations. Eluent anions at the top of the column are displaced by sample anions immediately after injection of the sample. These eluent anions and the sample cations travel with the solvent front to the detector and appear as a peak. This peak is due to the high absorbance of the displaced eluent anions. The "pseudo" peak, sometimes called the eluent displacement peak, represents the time necessary for a non-retained component to travel the system, or the void volume, V_o, of the system. It is

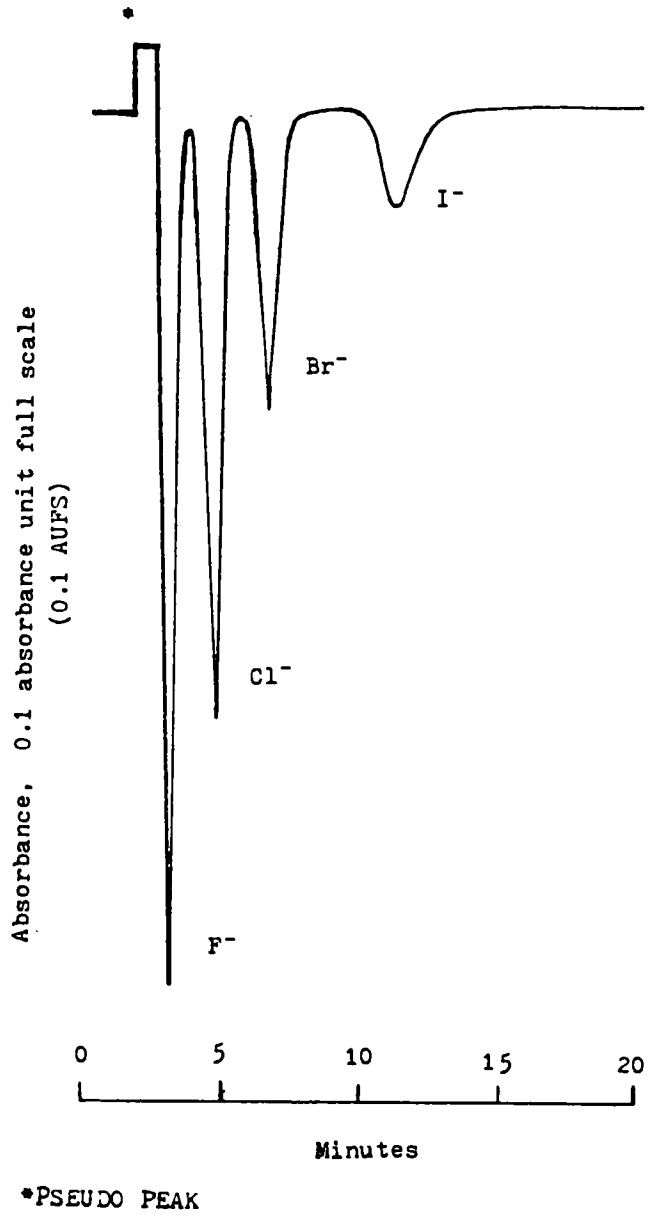

FIGURE 5--Separation of the Halides by the IPC Method

A Varian 5000 high performance liquid chromatograph was utilized with an AS Pellionex SAX anion exchange column to achieve this separation of the halides. 5×10^{-4} M sodium phthalate, pH = 7-8, at a flow rate of 1.0 mL/min was the mobile phase. A Perkin-Elmer LC-55 absorbance detector was set at a detector wavelength of 275 nm and a sensitivity of 0.1 AUFS. The sample was a 20 µL injection of NaF, NaCl, NaBr, and NaI, each at a concentration of 1 mg/mL.

analogous to the air blip often seen in gas chromatography. The quantity V_o is subtracted from sample elution volumes to obtain corrected elution volumes for each sample species.

One major advantage to IPC is the simplification of instrumentation. The second column in the original ion chromatograph was removed as was the associated tubing. Removal of this extra hardware decreased the void volume of the system. Decreased band broadening and better resolution and detection limits are the result of removing the suppressor system, which acted as a mixing chamber for the sample solute zones. The instrumentation shown in Figure 6 is analogous to that of many basic and readily available high performance liquid chromatographs. Applying IPC to existing equipment is another advantage in that an initial expense may be the purchase of a single ion exchange column for $300 instead of a complete ion chromatograph.

Quantification of sample ions is dependent on how accurately the change in the absorbance signal can be detected for each trough. Eluent concentration is monitored for the baseline so the corresponding absorbance must be accurately determined. Using highly absorbing eluents as the monitored background gives a greater difference in signals as the sample elutes, and thus the troughs appear larger and are much easier to detect for lower concentration sample species. The concentration of the eluent must be maintained to give an absorbance greater than 1.0 a.u. to produce a highly absorbing eluent, and less than 2.0 a.u. to avoid problems of nonlinearity. The use of a highly absorbing eluent allows the concentration of the eluent to be quite dilute, a typical range being

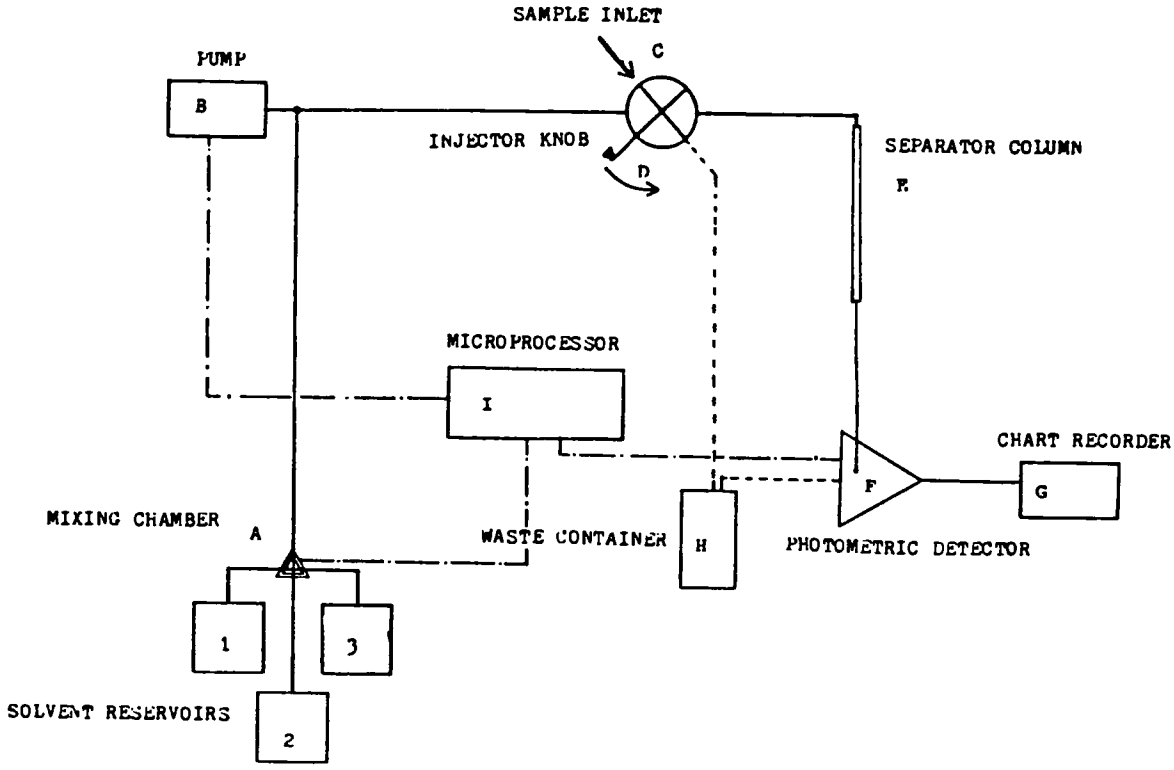

FIGURE 6--Instrumentation Utilized in IPC

The mobile phase is drawn from the solvent reservoirs, 1-3, mixed in the mixing chamber, A, and pumped via B to the injector system. The sample is introduced at the sample inlet, C, and sent to the head of the separator column, E, when the injector knob, D, is turned 45 degrees. The separated components are detected photometrically, F, and absorbance changes are recorded on the chart recorder, G. Excess sample and spent eluent are directed to the waste container, H. The eluent flow, solvent mixing, and sample component detection are monitored by the microprocessor, I. A comparison with the IC instrumentation in Figure 2 shows the suppressor column and its associated tubing have been removed and the conductivity detector has been replaced by a photometric detector.

1×10^{-2} to 1×10^{-4} molar solutions. Values for the molar absorptivity, E, should be from 100 to 1000 L/cm-mole to accomodate this concentration range. However, there are lower limits that must apply to the choice of eluent concentration. If the eluent is too dilute less efficient exchange occurs resulting in longer run times. This in turn leads to band spreading and lost sensitivity. Detector wavelength can be adjusted to keep the absorbance signal at an optimum. Also, by changing the detection wavelength a variety of eluent concentrations become available for use because the corresponding absorbance is a function of the monitored wavelength.

In order to study the system completely all variables of the mobile phase preparation must be optimized to give the most efficient separation. Also, all parameters on the HPLC instrument must be studied for optimization of the method. It is more practical to change the mobile phase variables than those of the stationary phase. Usually, a column is pre-selected knowing its specific characteristics, such as its packing and functionality, exchange capacity, dimensions, and cost.

There are two requirements of the eluent: it must be highly absorbing, having an absorbance value greater than one, and it must have little change in absorbance at the maximum wavelength or operating wavelength. An ultraviolet-visible absorbance spectrum will give the wavelengths at which to operate the detector for the most sensitive measurements. If the detector is not a variable wavelength type, an absorption spectrum will give the value of absorbance at the working wavelength of the detector, 254 nm or 280 nm, so the eluent concentration may be properly adjusted. It

is important to know if the eluent absorbs strongly enough in order to record peaks with adequate sensitivity.

To create a smooth baseline the operating wavelength is chosen on a plateau region of the absorption spectrum in order to minimize error from wavelength drift in the instrument. If an operating wavelength occurred on a slope of the absorption spectrum of the eluent in question, then slight changes in the operating wavelength due to the detector will result in changes in the baseline absorbance. The determination of sample species will not be accurate unless the wavelength changes very little. However, this baseline drift should not be a problem as most detectors have very good wavelength accuracy and reproducibility.

The distribution of eluent ion species may be pH dependent, so it follows that the selectivity of the separation and the retention time of each sample species are also pH dependent. Changing operating pH values is illustrated in values of the relative selectivity factor, or capacity factor, k' (29). At various pH values some species will only be partially ionized and move through the column rather quickly. Species with higher charges have greater interactions with the charged site on the resin and stay on the column longer. Therefore, a change in eluent pH will also change the retention times of each solute and the relative selectivity of the method.

By varying the eluent concentration and recording the elution volume of the solutes of interest for a given sample, an elution order plot may be constructed. Plotting corrected elution volume versus the eluent concentration shows the inverse relation of these two quantities (30). The elution volume of a particular

solute decreases upon increasing the concentration of the eluent. Other information such as predicting elution volumes and defining crossover regions, where two species elute at the same time, is obtained from these plots.

Reproducibility, or the lack of it, can be seen easily by making duplicate injections of each sample. If peaks vary in retention time or shape while using the same aliquot of mobile phase, then the entire system is not equilibrated. Some experimental or instrumental parameter may be changing during the experiment and the reasons for this behavior must be investigated. A check of the column efficiency is easily accomplished by injecting the mobile phase onto the column. A stable baseline should result and if not, any radical behavior must be investigated.

Finally, the sensitivity of the method can be visualized by constructing a standard plot of peak height or peak area versus the sample concentration injected. The result should be a linear plot which can then be used as a calibration curve for studying samples of unknown solute concentrations. Extrapolation to the ordinate shows the limitation of the method at a given attenuation setting for quantification of a given solute.

EXPERIMENTAL

The instrumentation utilized for the HPLC experiments presented in this paper consisted of a Varian 5060 liquid chromatograph (LC), a Rheodyne Model 7125 injector, and a Nucleosil SB 10 μm ion exchange column from Alltech-Applied Science containing trimethylamine functional groups. The LC was equipped with three solvent lines although only two were used at a time, a mixing chamber, and a high pressure pump. The microprocessor controlled solvent mixing to give the desired eluent. This system enabled the user to make rapid changes in eluent composition or concentration. The injector system, where the sample is introduced into the LC, utilized a 100 μL sample loop. Although there are many sizes of sample loops ranging from 5 μL to 5 mL, the 100 μL sample loop was the only size used. The method of filling the loop completely with a 250 cc syringe with a blunt ended needle was used for reproducible sample introduction.

The variable wavelength Varian Aerograph Model UV-100 detector was used for photometric detection of the ionic species and the strip chart recorder was a Fisher Recordall Series 5000. Absorbance spectra were taken on a Perkin-Elmer 552A UV-VIS Spectrophotometer equipped with a Perkin-Elmer 561 Model Recorder. The sample cells were 1 cm quartz cells using distilled water as the blank.

Commercially available reagent-grade chemicals and HPLC-grade water were used to prepare the copper (II) nitrate, trimesic and phthalic acid eluents. The pH was adjusted using a calibrated pH meter and 1 molar solutions of sulfuric, nitric, or

hydrochloric acids and sodium hydroxide. These solutions were prepared from the concentrated acids and the reagent-grade pellets. Stock solutions of 1000 ppm iodide ion and other common anions were prepared from the sodium or potassium salts and doubly distilled water. Standard solutions were obtained by diluting aliquots of these stock solutions. The ion exchange column was first equilibrated with the eluent to be used by maintaining a pump flow rate of 1.0 mL/min for 45 minutes. This column was regenerated at the end of each day's use by pumping a 30:70 mixture of HPLC grade methanol:water at 1.0 mL/min pump flow rate for 30 minutes.

Instrument conditions for optimizing the quantification of iodide ion were established by injecting standard samples in the concentration range of 200 ppm to 1 ppm I^- and varying instrument parameters. The I^- peak was identified by retention time measurements with respect to standard solutions. Peak areas were determined by the height times width at half-height method. The calibration data was to be obtained from a final set of conditions: eluent concentration, eluent pH, eluent flow rate, operating wavelength, and recorder sensitivity.

RESULTS AND DISCUSSION

In developing a liquid chromatography method to perform a specific analysis many variables must be taken into consideration. The LC instrumentation, particularly the analytical column, must be maintained and regularly inspected. If any component of the system, including the mobile phase or eluent, is not properly monitored correct analytical results may not be achieved. In this investigation three different aqueous eluents were pumped through the silica-containing ion exchange columns. The result was damage to the columns which was evident by the poor shape of the chromatographic peaks in the output. Data for each eluent system is discussed and tabulated in order to explain the reasons for the column degradation and to compare the three eluents in elution strength for the separation of iodide ion. Recommendations for optimizing the detection of iodide ion while avoiding column damage are also given in this discussion.

Copper (II) nitrate trihydrate, $Cu(NO_3)_2 \times 3H_2O$, was the first eluent investigated. The literature suggests its use in a tandem cation-anion determination (31). By connecting a cation exchange column in-line directly after the anion exchange column, both cations and anions could theoretically be separated and detected using this eluent. The anions of the sample would interact with the anion exchange functional groups in the first column and each would be retained for a length of time. The cations of the sample would pass through the anion exchange column to the cation exchange column to be separated according to the different

affinities each cation had for the stationary phase. After passing through both columns the sample cations and anions would be separated and passed to the detector. A tandem cation-anion determination such as this would greatly increase the method's usefulness.

The copper nitrate salt was deemed an appropriate eluent for indirect photometric chromatography because nitrate anions and copper (II) cations absorb in the UV-VIS region. Copper nitrate can exchange nitrate ions for anion chromatography or copper (II) ions for cation chromatography. Both are highly absorbing ions which would make copper nitrate a particularly useful eluent for a total ion determination utilizing both anion and cation exchange columns.

A wavelength study from 254 to 245 nm using the Varian 5060 HPLC was conducted to check the efficiency of the column and to "monitor" the system. 5×10^{-3} molar copper nitrate was the eluent and 100 μL injections of distilled water served as the sample. The operating wavelength was changed for each injection of H_2O to observe the effect on the sensitivity of the method as manifested in the size and shape of the chromatographic "valleys" or troughs produced. One major component was found to have a retention time of 8.3 minutes. It was not necessary to identify the trough at this point because the column reproducibility was being investigated; however, some impurity was expected to be producing the "observed" trough. The quality of each chromatogram was noted as the baseline was smooth and the one component had a reproducible retention time, R_t = 8.3 min. Each chromatogram was reproducible so the system was

assumed to be functioning properly and further experiments were undertaken.

At this point the unidentified trough, typically referred to as a peak, was thought to be due to the presence of sulfate ion which was introduced in the system in the preparation of the eluent. To dissolve the copper nitrate a small amount of sulfuric acid was added although the CRC Handbook claims copper (II) nitrate trihydrate as soluble to the extent of 137.8 g in 100 mL cold water (32). The predominant ionic species in solution were Cu^{2+}, NO_3^-, and HSO_4^- because the system was equilibrated with 5 x 10^{-3} molar copper nitrate eluent containing 2 x 10^{-4} molar sulfuric acid. Eluent anions at the top of the column are displaced by sample anions immediately after injection of the sample. These eluent anions and the sample cations travel with the solvent front to the detector and appear as a peak. No other ionic species should have been present in the distilled water or the eluent to produce the trough. During elution the sulfate ions from the eluent were exchanged by the active sites on the exchange resin and retained on the column for a few minutes. The detector recorded the change in eluent concentration as a negative peak. The identity of the sulfate peak was confirmed by separate injections of HPLC H_2O and 100 ppm sodium sulfate. The noted retention time for each trough was 8.3 min consistently. Copper (II) nitrate which is dissolved with the aid of sulfuric acid gives this "system peak" in each chromatogram which may interfere with subsequent solution analyses. Under the same conditions injections of 10 ppm potassium iodide gave the iodide ion retention time at 15.4 min. At an eluent flow rate of 2.0 mL/min the elution volume of iodide ion, V_e, is 30.8 mL

of the 5×10^{-3} molar copper nitrate eluent. Figure 7 shows two chromatograms of these injections which helps to illustrate the identification of the "system peak." The troughs appear as positive peaks because polarity was switched on the recorder to give the opposite but more familiar orientation.

The conditions used for the determinations in Figure 7 are not suitable for very sensitive determinations of iodide ion. Using these conditions a detection limit of 2 ppm I^- may be achieved. The sensitivity was set at a low 0.01 au/mv. It was not beneficial to increase to a more sensitive setting because doing so inherently increased the noise of the system. A small signal from a sample containing less than 2 ppm I^- would be muddled or lost in the large background noise. For environmental water samples the expected signals are sub-part per million levels of iodide ion. According to Kirk-Othmer (33) the maximum contaminant levels of inorganic chemicals for drinking water in the United States is 0.05 mg/L which is equivalent to 0.05 ppm I^-. Also reported is the value of 60 µg/L as a typical level for iodide ion occurring in seawater which is equivalent to 0.06 ppm I^-. Conditions for the determination of iodide ion would have to be refined for detection of these part per billion concentrations.

The preparation of the eluent must be standardized to avoid day-to-day variations in the output. Table III shows the conditions used in achieving total solution for sixteen 500 mL aliquots of copper nitrate. The data shows that acidifying copper nitrate with sulfuric acid gives non-reproducible results; the final pH varied from 2.38 to 5.05. The copper nitrate eluent was prepared by

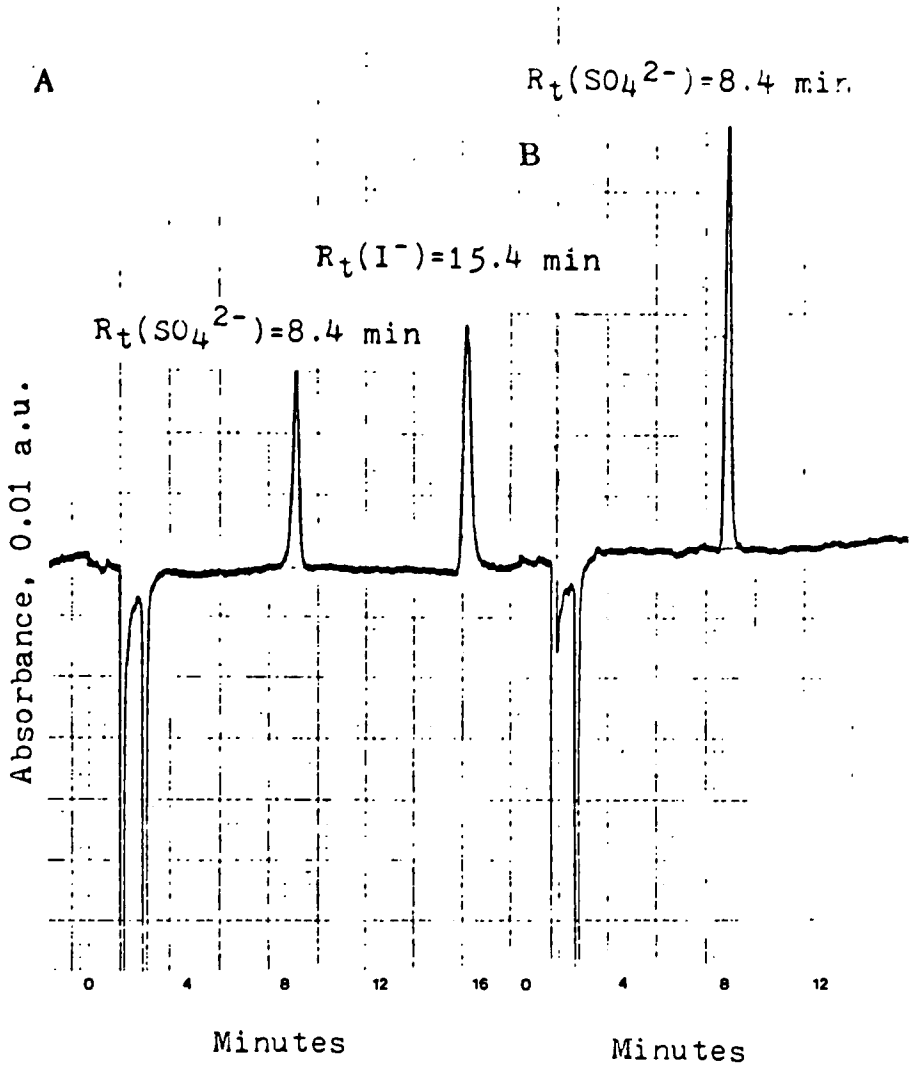

FIGURE 7--Examples of IPC Chromatograms

Eluent: 5 x 10^{-3} molar $Cu(NO_3)_2$
Flow Rate: 2.0 mL/min
Detector: 254 nm, 0.01 au/mv

Chromatogram A
Sample: 100 µL of 10 ppm KI
Retention Times, R_t:
Sulfate, 8.4 min
Iodide, 15.4 min

Chromatogram B
Sample: 100 µL of 100 ppm Na_2SO_4
Sulfate, 8.4 min

weighing out 0.58 g $Cu(NO_3)_2 \times 3H_2O$ into a 600 mL beaker equipped with a stirring bar, magnetic stirrer, and a calibrated pH meter. The initial pH was taken after adding 300 mL distilled water and ranged from 4.50 to 5.42 before the addition of any acid. Upon adding two or three drops of concentrated sulfuric acid the pH rapidly went down to 2.5-3.0, which later chromatograms show to be unacceptable as an eluent pH. The solution was transferred to a 500 mL volumetric flask and diluted and filtered before use as an eluent.

Table III

Standardizing Preparation of 5×10^{-3} molar $Cu(NO_3)_2$ Eluent

500 mL aliquot	pH^1	Added H_2SO_4*	pH^2	Stirring (min)
A	5.38	5%	3.74	180
B	5.42	5%**	3.00	20
C	--	3c	2.70	20
D	--	1c	3.28	20
E	4.60	5%	3.80	80
F	4.69	5%	5.05	960
G	5.30	0	--	1920
H	4.99	5%	4.35	150
I	5.28	5%	4.11	150
J	5.19	5%	4.05	70
K	4.84	5%	3.10	120
A2	4.85	5%	3.58	160
B2	--	3c	2.38	10
C2	--	2c	2.60	20
D2	--	1c	3.00	40
E2	4.50	5%	--	20

pH^1 represents the initial pH taken before addition of any acid

pH^2 represents the final pH taken before use as an eluent

* 5% H_2SO_4 was added until a change in the pH was registered on the pH meter

** 3 drops of concentrated H_2SO_4 were added using a disposable pipet

-- no pH readings taken

The pH differences are important to note. Slight changes in eluent pH will have a marked effect on the elution volume of the sample ions because the species present in solution change with variations in pH. An example is the case of copper (II) hydroxide, $Cu(OH)_2$, which undergoes this equilibrium:

$$Cu(OH)_2 \rightleftharpoons Cu^{2+} + 2OH^-;$$

$$K_{SP} = [Cu^{2+}][OH^-]^2 .$$

From the solubility product, $K_{SP}=4.8 \times 10^{-20}$ (34), it is found that a 5×10^{-3} molar copper nitrate solution will precipitate copper hydroxide at pH=5.49. If any pH changes are encountered during a run and hydroxides are precipitated in the column, irreversible damage may be the result.

The retention time of the iodide ion varies with the pH of the copper nitrate eluent. The retention times and elution volumes of iodide ion using different pH eluents are compared in Table IV. The lower pH eluent is stronger in eluting power but also contains system peaks which may interfere with the determination. Peak #3 in each chromatogram of Figure 8 travels to greater retention times with increasing eluent pH. An extraneous peak such as this one which varies with changes in pH may be due to a species present in only a given pH range. Examples of these species may include dissolved carbonate, bicarbonate and carbon dioxide.

Metals in aqueous solutions react in a variety of ways with the ionic species present, such as OH^- and H^+. In aqueous solutions copper complexes with water to form these species among others: $Cu(H_2O)_2(OH)_2$, $Cu(H_2O)_3(OH)^+$, $Cu(H_2O)_4^{2+}$ (35). The predominant

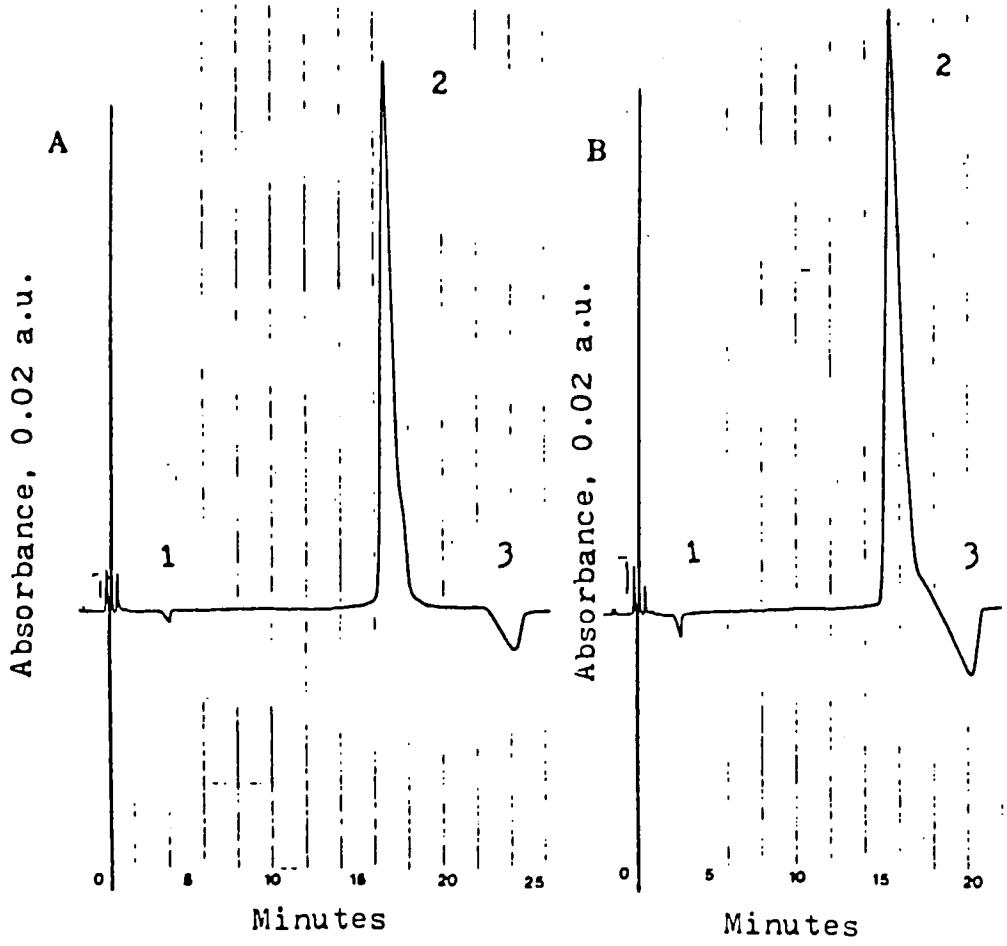

FIGURE 8--Peak Elution As a Function of Eluent pH

Eluent: 5 x 10^{-3} molar $Cu(NO_3)_2$
Sample: 100 µL of 100 ppm KI
Detector: 254 nm, 0.02 au/mv
Flow Rate: 3.5 mL/min

Chromatogram A

Eluent pH=3.07

Peak	R_t(min)
1	4.0
2	16.6
3	23.9

Chromatogram B

Eluent pH=2.88

Peak	R_t(min)
1	3.3
2	15.5
3	20.2

copper complex in aqueous solutions is $[Cu(H_2O)_6]^{2+}$ (36). Sometimes the conditions of a chromatographic run include one or more of these aqueous species whose presence is due to the composition and hydrogen ion concentration of the eluent. Peak #1 of each chromatogram in Figure 8 is an example of the system peak which may be a result of some anionic metal complex or dissolved gas. This small peak is visible at low pH but disappears at eluent pH=3.35 or greater.

Table IV

Comparison of Iodide Ion Elution Volumes*

Eluent pH	V_e(mL)**
2.88	54.3
3.07	57.4,58.1
3.35	65.5,65.1
3.64	67.6,67.6***
3.84	67.9,67.8***

*The elution volume, V_e, values given for all eluent pHs, except pH=2.88, represent two different trials. The average of the two values was used in further analyses.

**V_e(mL) = R_t(min) * Flow Rate, (mL/min)

Conditions:

Eluent ---------- 5 x 10^{-3} molar $Cu(NO_3)_2$
Sample ----- 100 µL injection of 100 ppm KI
Flow Rates ----------3.5 mL/min;
*** ----------3.0 mL/min.

The effect of the mobile phase pH on the capacity factor, or the relative selectivity factor, k', is shown in Figure 9. The relation of the capacity factor to retention times, or elution volumes is the following:

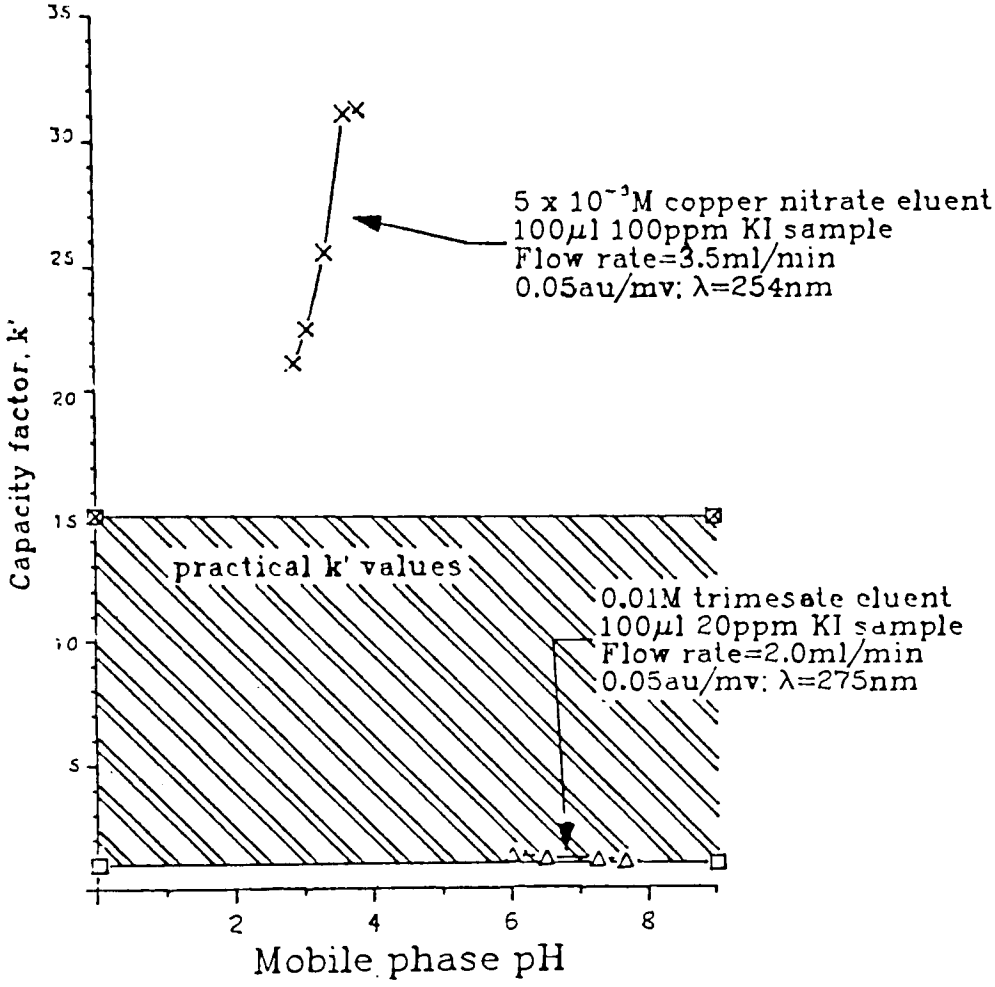

Figure 9--Effect of mobile phase pH on the system selectivity

$$k' = (R_t - R_v) / R_v$$

$$k' = (V_e - V_v) / V_v$$

where R_v is the retention time of the void disturbance and V_v is the void elution volume. The plotted values of k' are much higher than the optimal values, k'=3-8 (37). Interpreted as unnecessarily long retention times for the iodide ion, these large k' values must be decreased in order to achieve quantitative results within ten minutes. Analyses requiring longer retention times waste valuable instrument time, use more eluent, and exhaust columns faster than analyses for components with short retention times.

The capacity factor is defined as a function of the ratio of the volumes of mobile and stationary phases in the column (38). Improvements in the capacity factor are made by changing the mobile or stationary phases to adjust the time spent by the solute in either phase. Large k' values indicate the solute has a high affinity for the stationary phase and is manifested by a long retention time. To decrease the solute-stationary phase interaction the mobile phase polarity, pH, or ionic strength may be altered. Changes made to these mobile phase characteristics to allow the solute to have a stronger interaction with the mobile phase will result in a shorter retention time.

The strong base anion exchanger used as the stationary phase was not altered to attempt changing the capacity factor; however, the mobile phase could have been modified. The Nucleosil SB column has a working pH range of 1-9 but the eluent does not dissolve in distilled water alone. Some acid must be added to dissolve the

solid copper nitrate. Continued use of this acidic eluent can lead to problems of column degradation. The pH of 5 x 10^{-3} molar $Cu(NO_3)_2$ is in the range of 2.5-4.0. Figure 9 illustrates that k' decreases with decreasing pH. Therefore, pH is not an alternative in varying the conditions to change the selectivity using the copper nitrate eluent in this system. An increase in the ionic strength of the eluent or a higher concentration of the eluent could be used to shorten the retention time of iodide ion, but there would be problems of dissolving the copper nitrate solid completely.

The copper nitrate/sulfate system was not proven the best eluent in these studies. Completely dissolving the solid copper nitrate, the low pH eluent, and the long retention time of 15.4 min for I^- are problems with using this eluent. Therefore, a new eluent was investigated for the quantification and detection of iodide ion.

Organic acids have been proven as eluents for ion chromatography and also may be applied to IPC (39). From this family of compounds trimesic acid, or 1,3,5-benzenetricarboxylic acid, was chosen as an eluent. It is a triprotic carboxylic acid and has three proton transfer equilibria in aqueous solutions (40):

$$H_3A \rightleftharpoons H_2A^- + H^+; \quad K_1 = 7.59 \times 10^{-3}$$
$$H_2A^- \rightleftharpoons HA^{2-} + H^+; \quad K_2 = 1.29 \times 10^{-4}$$
$$HA^{2-} \rightleftharpoons A^{3-} + H^+; \quad K_3 = 2.00 \times 10^{-5}$$

Variations in pH change the relative concentrations of the species present in a solution of trimesic acid. The following definitions are useful to graphically display how the solution

composition is affected by pH. The sum of all trimesic-containing species is represented by C,

$$C = [H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}].$$

The concentration of a particular species can be found by defining it as a fraction of the whole, a, where the sum of all a's is unity:

$$a_0C=[H_3A],\ a_1C=[H_2A^-],\ a_2C=[HA^{2-}],\ a_3C=[A^{3-}]$$
$$a_0 + a_1 + a_2 + a_3 = 1$$

By combining the equilibrium expressions for the dissociation of trimesic acid and the mass-balance equation,

$$C = [H_3A] + \frac{K_1[H_3A]}{[H^+]} + \frac{K_1K_2[H_3A]}{[H^+]^2} + \frac{K_1K_2K_3[H_3A]}{[H^+]^3}$$

Solving this equation for $[H_3A]$ and inserting the new expession into the definition of a_0, this is the result:

$$a_0 = \frac{[H^+]^3}{[H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3}$$

Similar expressions are achieved for each "a" term which contain only the equilibrium dissociation constants and the hydrogen ion concentration. The denominator is represented by D in the following three equations because the denominator is the same as that for the previous expression.

$$a_1C = K_1[H^+]^2 \ / \ D$$
$$a_2C = K_1K_2[H^+] \ / \ D$$
$$a_3C = K_1K_2K_3 \ / \ D$$

Therefore, for any given value of pH, i.e. $[H^+]$, a set of "a values" is calculated. By calculating each of these values for the pH range from 0 to 14 and by plotting "a" vs. pH, the distribution of the trimesic acid species is illustrated as seen in Figure 10. At a glance it is possible to tell which species exist at a given pH and the relative concentrations of each.

The fully protonated acid predominates in solution at pH=0-2 and a mixture of all four species exists at pH=3-4. The completely ionized trivalent trimesic acid, or the trimesate ion, exists primarily at pH>6. Harming the stationary phase is not a major problem at an operating pH=6-7 because it is in the middle of the acceptable range for the Nucleosil column. The samples to be quantified are aqueous with pH=7 so there is little difference between sample and eluent pHs. Also, the totally deprotonated specie should be the most powerful eluting specie because it contains three sites with which to bind to the resin. This does not mean all three sites will bind at the same time because that is sterically impossible, but rather, the probability of binding to the resin is greatly increased. This great affinity for the resin sites will elute anions from the column quickly so trimesate ion is expected to be a more powerful eluent than nitrate ion.

The absorbance of the trimesate ion was checked using the Perkin-Elmer 552A UV-VIS spectrophotometer and a 1×10^{-2} molar solution of the acid. 0.1 molar NaOH was added to the solution dropwise to obtain several aliquots with different pH values. The corresponding absorbance spectrum of each aliquot showed little variance of absorbance with pH. Three plateau regions are noted in

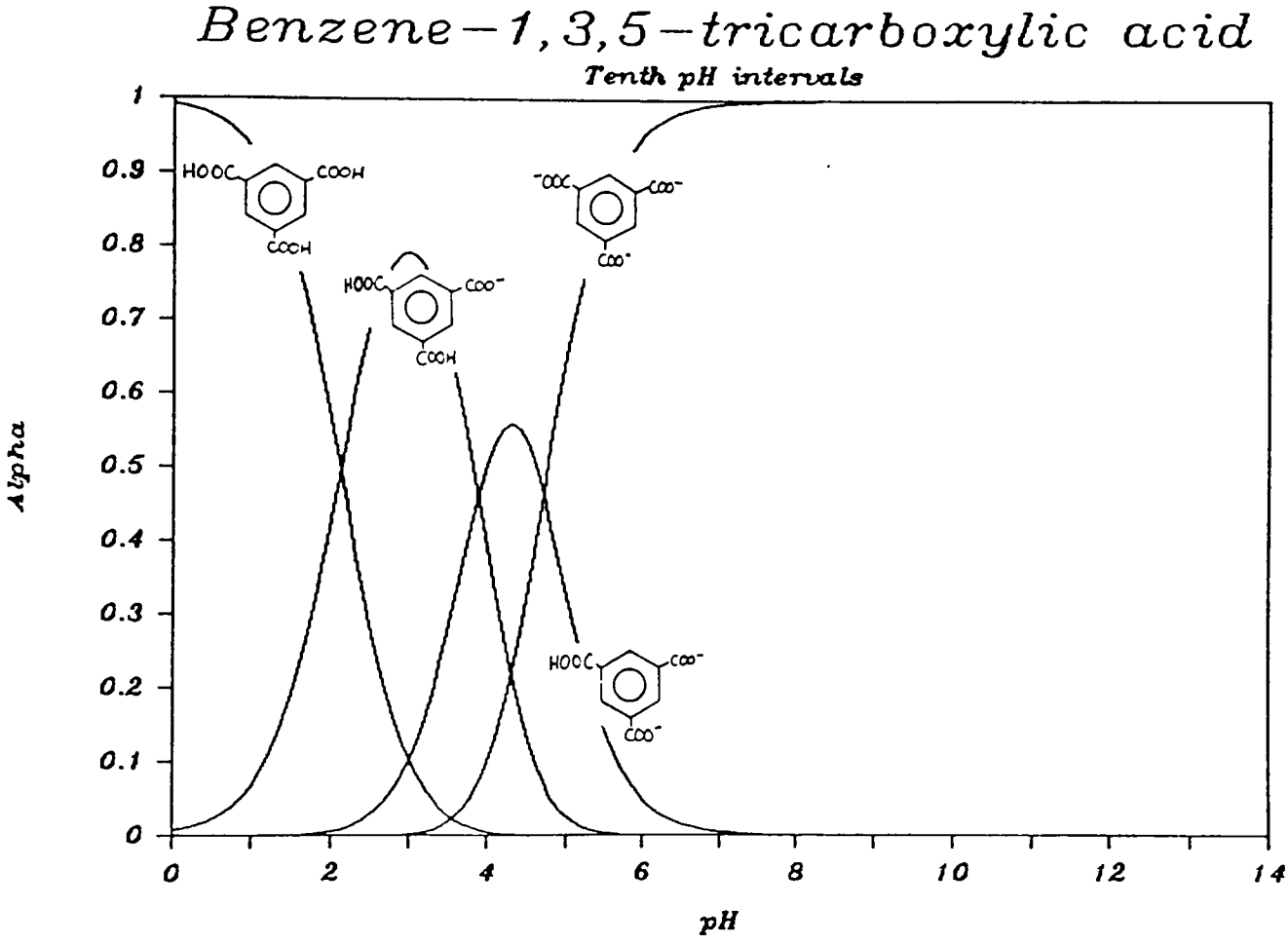

FIGURE 10--Distribution of the Trimesic Acid Species as a Function of pH

the absorbance spectra: 288-291 nm, 279-281 nm, 275-276 nm. A 1 x 10^{-2} molar solution of trimesic acid gave A=1.6 at 290 nm.

A sensitivity study was completed by injecting 100 μL samples of 20 ppm NaI into the HPLC using 1 x 10^{-2} molar trimesate, pH=6, as the eluent and varying the wavelength for each injection. The retention time of I^- was consistent at 3.7 min. Peak height increased with decreasing wavelength and the most sensitive wavelength was found to be 275 nm. The stronger eluting power of trimesic acid over copper nitrate was proven as the retention time was well within the limit of a 10 minute determination. The elution volume was also much less, V_e=7.4 mL of the 1 x 10^{-2} molar trimesic acid eluent. Figure 11 gives two examples of chromatograms obtained with the Varian 5060 LC that use 0.01 molar trimesate eluent with different samples and instrument conditions. Chromatogram B gives a larger signal than chromatogram A because the detector is set at a more highly absorbing wavelength and a higher sensitivity.

The recorder sensitivity setting were varied for identical injections to check the noise of the system at sensitive recorder readings. The more sensitive 0.01 au/mv (absorbance unit/millivolt) setting shows a greater deviation of peak area measurements than the less sensitive 0.05 au/mv. Assuming that the linear relation of sample concentration to peak area holds when the detector sensitivity is changed, a detection limit may be achieved of 2 ppm I^- utilizing the following conditions: 1 x 10^{-2} molar trimesic acid eluent, pH=7, 2.0 mL/min pump rate, 275 nm detection wavelength, and 0.01 au/mv sensitivity setting.

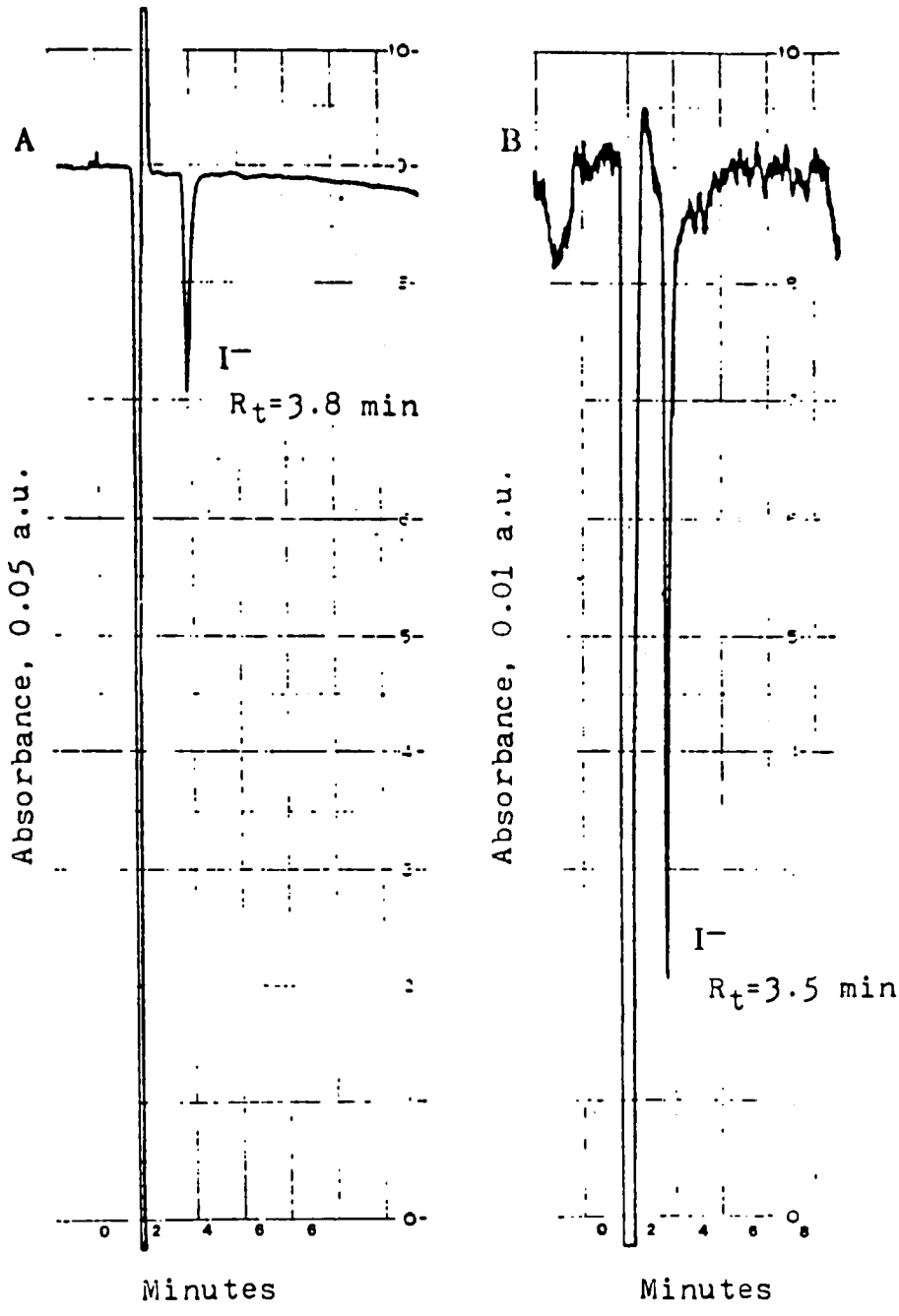

FIGURE 11--Chromatograms Obtained with the Varian 5060 Liquid Chromatograph

Chromatogram A
Eluent: 0.01 molar trimesate, pH=6.00, 2.0 mL/min
Sample: 100 μL of 20 ppm KI
Detector: 290 nm, 0.05 au/mv

Chromatogram B
Eluent: 0.01 molar trimesate, pH=7.25, 2.0 mL/min
Sample: 100 μL of 10 ppm KI
Detector: 275 nm, 0.01 au/mv

In an attempt to detect the iodide ion content of tap water samples using these optimized conditions, irreproducible chromatograms were obtained. The jagged baseline showed little equilibration of the system. Sensitivity settings from 0.01 au/mv to 1.0 au/mv showed no equilibration of the baseline although the problem was greatest at the most sensitive 0.01 au/mv. Hardware of the HPLC was investigated for problems such as collection of salts on filter frits and leaks around tubing connections. The flow cell of the detector and the stainless steel tubing of the system were cleaned by removing the column and replacing it with a length of stainless steel tubing. The system was purged with HPLC grade water, followed by 20% nitric acid, HPLC water, and HPLC grade methanol. Inlet filter frits contained in the eluent reservoirs were cleaned in the same manner and a leak in the piston seal assembly was repaired, but all failed to remedy the detection problem. The column was deemed at fault. An identical column was ordered, but the one received had 5 μm particles instead of the requested 10 μm size. The smaller particle size would help to give a smaller selectivity factor to the determination, so the new column was used in attempting to optimize conditions to lower the detection limit.

Preliminary tests using the 1×10^{-2} molar trimesate eluent showed the same jagged baseline as seen with the old 10 μm column. Range settings more sensitive than 1.0 au/mv showed a very strong drift and little equilibration of the baseline even after pumping eluent at 2.0 mL/min for an hour. The detector flow cell may have had miniscule air bubbles introduced upon changing columns which would explain the unsteady baseline signal. Air bubbles that are

trapped in the flow cell bounce around or otherwise obstruct the detector path which results in erratic output. The flow cell assembly was thoroughly cleaned as before by removing the column and purging the system to remove any air bubbles present. The eluent was filtered through a medium fritted crucible and degassed in a sonicator to remove dissolved gasses which may also have introduced air bubbles into the system, but to no avail; the unstable baseline was still present.

One last parameter, column temperature, may be controlled to effect a better or more reproducible separation. A great percentage of articles found in the literature incorporate the use of either a column oven or a water jacket and constant temperature water circulator. These temperature control devices ensure that ion exchange takes place as the result of an equilibrium process occurring at a given temperature. As a result of investing in one of these devices the monitored eluent concentration level remains a constant and one more variable of the system is controlled.

A major requirement of this indirect method of detection is that a highly absorbing eluent be used to emphasize the difference between the eluent and sample species' absorbances. However, this difference cannot be too large because small variations in the monitored eluent concentration will be exaggerated as an unsteady baseline at the output. At a sensitivity setting of 0.05 au/mv, which is the start-up condition for the Varian 5060 HPLC, an injection of 60 ppm KI was not detected using the 1×10^{-2} molar trimesate eluent at 254 nm. At this point the eluent absorbance was too large to show the small decrease in absorbance as the iodide ion

eluted. This type of baseline noise is absent in direct modes of detection because the monitored baseline signal is non-absorbing and does not produce a large background level.

The concentrated eluent may have crystallized in the inlet filter frits contained in the eluent reservoirs. Trimesic acid is slightly soluble in water, 0.24 g/100mL H_2O (41). Upon cleaning these filters an insoluble, white solid was extruded. At irregular intervals the salt collected here may have been dissolved into solution and carried though the system to give varying levels of absorption at the output. In order to guard against this happening one solution to the problem would be to regularly clean the filter frits. The frits were cleaned and purged with HPLC grade methanol and isopropanol which lessened but did not eliminate the detection problem.

A concentrated eluent is not the only possible source for this unsteady baseline. The trimesate eluent was diluted to 1×10^{-4} molar and the baseline problem continued, but to a lesser extent, even after repeated attempts to clean and equilibrate the column. 1×10^{-2} molar HCl was pumped through the system to remove the trimesate. Both the anions and cations were useful in removing the organic acid counter ion. The H^+ ions would combine with the trimesate ion to form the totally protonated acid which then would leave the system as it had no interaction with the charged sites on the column. This reaction was enhanced by the available Cl^- counter ions, which took the place of the trimesate ion to become the new counter ion.

The trimesate would have been cleaned from the column rather quickly assuming only ion exchange interactions were occurring.

This was not the case as a pump flow rate of 1.0 mL/min for 60 min gave a large continuous drift of the baseline in the negative direction. The absorbance of the monitored effluent decreased very slowly. The trimesic acid was held on the column tenaciously, which suggested that some adsorption process was also taking place.

At this point a new eluent, potassium hydrogen phthalate, KHP, was investigated. KHP is the salt of the intermediate form of phthalic acid and is readily available as a primary acid standard. At pH>5 the divalent phthalate ion predominates in solution and should be fairly strong in eluting power. Using the given proton transfer equilibria, Figure 12 was constructed which shows the distribution of phthalate species as a function of pH.

$$H_2A \rightleftharpoons HA^- + H^+; \quad K_1 = 1.12 \times 10^{-3}$$
$$HA^- \rightleftharpoons A^{2-} + H^+; \quad K_2 = 3.91 \times 10^{-6}$$

The absorbance spectrum gave the optimum wavelength to be 266 nm with an absorbance of 1.1 for a 1.5×10^{-3} molar KHP solution at a pH of 6.3. Using this solution at a flow rate of 3.0 mL/min gave the retention time and elution volume for an injection of 80 ppm KI: R_t=14.6 min, V_e=43.8 mL.

A backpressure of 270 ATM showed the 3.0 mL/min flow rate to be the upper limit for this aqueous eluent because the maximum pressure on the column cannot exceed 350 ATM. Using higher flow rates exceeded the maximum backpressure allowed and the instrument automatically stopped the pump. An increase in flow rate was not advantageous in attempting to shorten the iodide ion retention time. Higher concentrations of KHP were tried but the baseline problem only became worse.

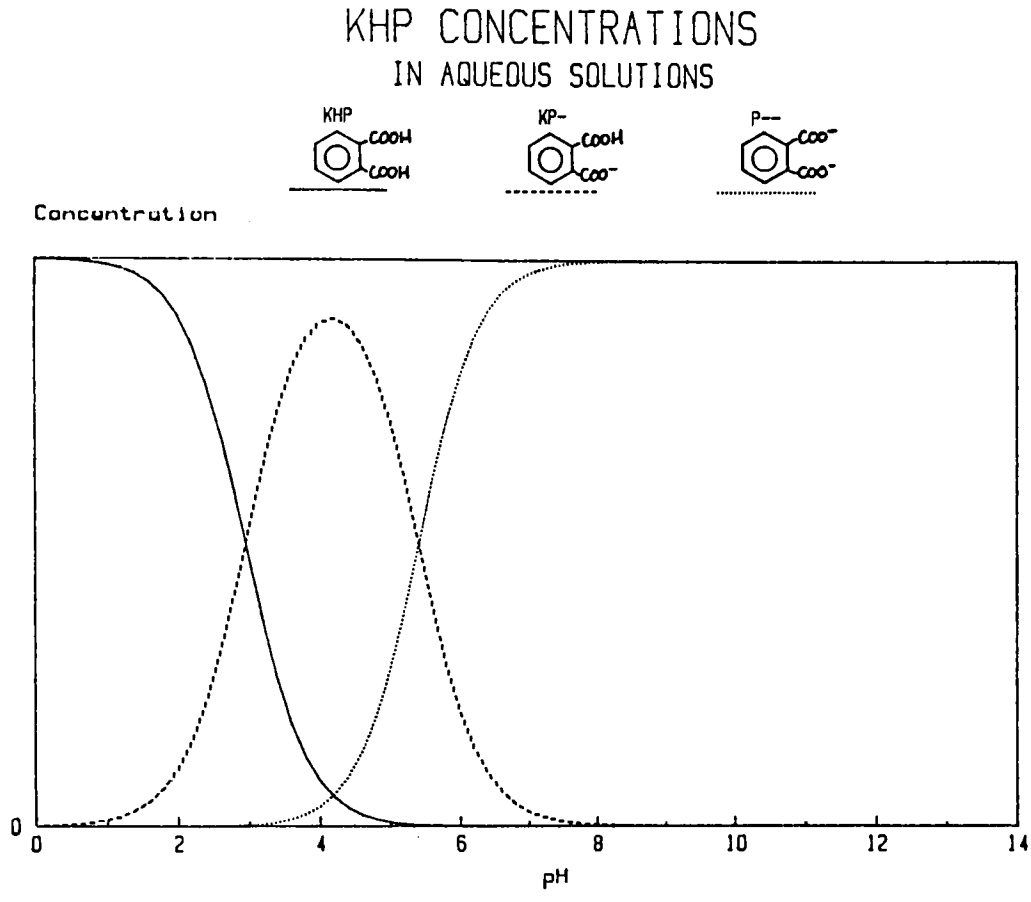

FIGURE 12--Distribution of the Phthalic Acid Species as a function of pH

Monitoring the degradation of the column was possible by running a normal mode ion exchange separation of two nucleotides. Using available biochemicals the best conditions for a column efficiency test were found to be a 100 μL injection of 0.025 mg/mL each of guanosine-5'-phosphate (GMP) and indosine-5'-phosphate (IMP) as the sample, 0.2 molar KH_2PO_4, pH=6.8 as the eluent, a flow rate of 2.0 mL/min, an operating wavelength of 254 nm, and a recorder sensitivity of 0.5 au/mv. The two major components eluted at 5.8 and 9.0 min with the new 5 μm column, representing IMP and GMP, respectively. This test run was repeated after every 2-3 liters of eluent had been pumped through the column.

Irreversible column damage is illustrated in comparing the chromatograms of Figure 13. To compare the efficiency of the new column to the same Nucleosil SB, 5 μm column after pumping eluent through the system, the number of theoretical plates, N, was calculated. The number of theoretical plates is simply the number of times that equilibration occurs between the mobile and stationary phases. In general a chromatographer strives for a high value of N to show that there has been efficient contact between the mobile and stationary phases. Assuming Gaussian shaped peaks are the result of this chromatography the following can be used with experimental data in determining separation efficiency:

$$N = 16(R_t/t_w)^2,$$

where t_w is the width of the peak of interest at the baseline. Chromatogram B of Figure 13 shows a decrease of an order of magnitude in N from chromatogram A. Utilizing this column for

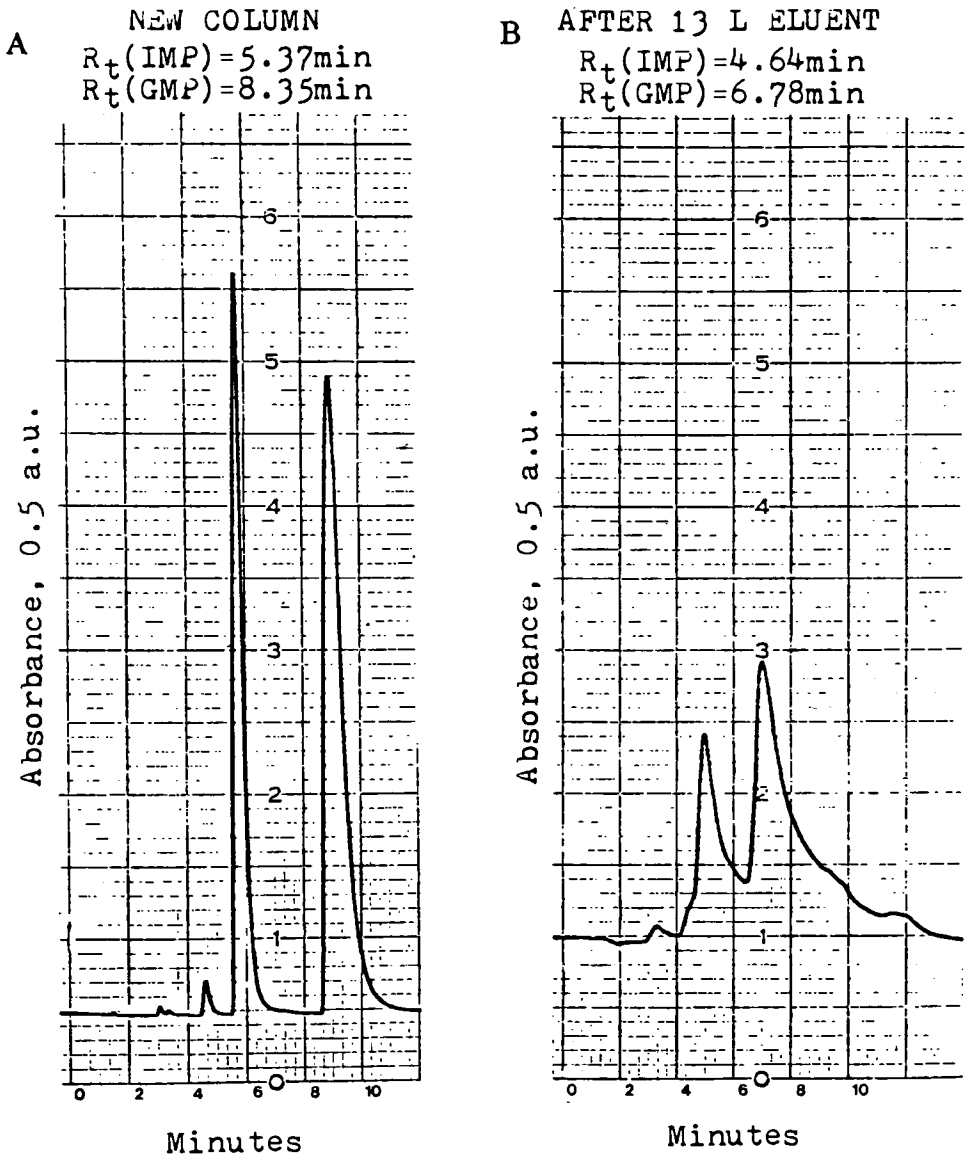

FIGURE 13--Final Chromatograms Showing Irreversible Column Damage

Eluent: 0.2 molar KH_2PO_4, pH=6.8
Flow Rate: 2.0 mL/min
Sample: 100 µL of 25 µg/mL of IMP and GMP
Detector: 254 nm, 0.5 au/mv
Number of Theoretical Plates, N

Chromatogram A
IMP, N=160
GMP, N=100

Chromatogram B
IMP, N=13
GMP, N=16

quantitative work would have been futile at this point. As previously mentioned several clean-up procedures were tried, but none worked well enough to permit further analytical work on this column. Both the eluent and the sample molecules may interact irreversibly with the column packing which would result in output like that of Figure 13.

The styrene-divinylbenzene matrix of the resin is aromatic in character, as are the trimesate and phthalate eluents. Even at the correct pH for each eluent to be fully ionized, there may be enough aromatic character in the eluent molecule for it to associate with the resin matrix. Some eluent molecules will be adsorbed to the surface of the resin bead making it more difficult for ion exchange to take place.

The iodide ion being injected as a sample may be held on the resin strongly. When dilute eluents were used, on the order of 0.5-2.0 x 10^{-3} molar, peaks were not registered in 30-40 minutes for injections of 100 ppm KI. This suggests a very strong interaction of I^- with the column packing. Iodide ion is the largest of the common halogen series and is expected to elute last in the usual ion exchange series after F^-, Cl^-, and Br^-. I^- is very polarizable due to its large van der Waals radius which would allow for strong interactions with the charged sites on the resin or with the available charged sites on the eluent molecules. These interactions may be stong enough to act as permanent bonds as evidenced by the lack of sample peaks with dilute eluents and by the inability of the cleaning procedures to remove the adsorbed species.

Table V summarizes the separating conditions used to achieve the tabulated detection limits and elution volumes for iodide ion.

Problems were encountered with each eluent and results indicate the copper nitrate, trimesate, and phthalate eluents were not effective in determining iodide ion in aqueous solutions below 2 ppm I^-.

Table V

Optimum Separation Conditions For Each Eluent Used With the Nucleosil SB Ion Exchange Columns

Eluent	$Cu(NO_3)_2$	Trimesate	Phthalate
Concentration (molar)	5×10^{-3}	1×10^{-2}	8×10^{-2}
Wavelength (nm)	245	275	266
Absorbance (au)	1.2	1.6	1.1
pH	4	6	7
R_t (I^-) (min)	15.4	3.7	14.6
Flow Rate (mL/min)	2.0	2.0	3.0
V_e(I^-) (mL)	30.8	7.4	43.8
Detection Limit	2 ppm	2 ppm	5 ppm

Future possibilities exist for using the IPC method in this application if some suitable eluent is found for the styrene-divinylbenzene based column or if the stationary phase is changed. In looking for a new eluent two requirements should be kept in mind: 1) the eluent must absorb at a wavelength compatible with the detector of the system, preferably A>1.0, and 2) the eluent must contain as little aromatic character as possible but still retain its absorbance to be effective as an eluent in the indirect mode of detection.

Although many possible eluents can still be investigated, a different stationary phase may prove the best alternative to using the SSS/DVB anion exchanger. Schmitt and Pietrzyk (42) studied the ion exchange separation of anionic analytes on an alumina stationary phase. The selectivity of the column for iodide ion was shown to be much better than the resin based column. The elution order of anions was found to be almost a reverse of the order observed using the silica-based column. Elution volumes less than 5 mL eluent were required for the elution of the iodide ion as compared to the values in Table V.

REFERENCES

(1) Peters, D. G.; Hayes, J. M.; Hieftje, G. M. "Chemical Separations and Measurements, Theory and Practice of Analytical Chemistry"; W. B. Saunders Company: Philadelphia, PA, 1974, p. 231, 246, 248.

(2) Meites, L.; Ed., "Handbook of Analytical Chemistry", 1st ed.; McGraw-Hill Book Company: New York, NY, 1963, Sect. 3, 5, 12.

(3) Johnson, E. L.; Stevenson, R. "Basic Liquid Chromatography"; Varian Associates, Inc.: Palo Alto, CA, 1978, p. 116.

(4) Pietrzyk, D. J.; Frank, C. W. "Analytical Chemistry", 2nd ed.; Academic Press: New York, NY, 1979, p. 479-481.

(5) Peters, D. G.; et al. p. 580.

(6) Peters, D. G.; et al. p. 583.

(7) Willard, H. H.; Merritt, L. L., Jr.; Dean, J. A,; Settle, F. A., Jr. "Instrumental Methods of Analysis", 6th ed.; D. Van Nostrand Company: New York, NY, 1981, p. 547.

(8) Dorfner, K. "Ion Exchangers, Properties and Applications", Coers, A. F., Ed., Ann Arbor Science Publishers, Inc.: Ann Arbor, Michigan, (1972), p. 17.

(9) Pohl, C. A.; Johnson, E. L. J. Chromatogr. Sci. 1980, 18, 442-452.

(10) Willard, H. H.; et al. p. 545.

(11) Small, H.; Stevens, T. S.; Bauman, W. C. Anal. Chem. 1975, 47, 1806.

(12) Small, H.; et al. p. 47, 1801.

(13) Willard, H. H.; et al. p. 548.

(14) Small, H. Anal. Chem. 1983, 55, 235A.

(15) Small, H. p. 239A.

(16) Stevens, T. S.; Jewett, G. L.; Bredeweg, R. A. Anal. Chem. 1982, 54, 1206.

(17) Dasgupta, P. K. Anal. Chem. 1984, 56, 96.

(18) Dasgupta, P. K. Anal. Chem. 1984, 56, 103.

(19) Small, H. p. 237A.

(20) Peters, D. G.; et al. p. 528.

(21) Wescan Instruments, Inc., 3018 Scott Rd., Santa Clara, CA, 95054.

(22) Gjerde, D. T.; Fritz, J. S.; Schmuckler, G. J. Chromatogr. 1979, 186, 509.

(23) Gjerde, D. T.; Schmuckler, G.; Fritz, J. S. J. Chromatogr. 1980, 187, 35.

(24) Cortes, H. J. J. Chromatogr. 1982, 234, 517.

(25) Heckenberg, A. L.; Haddad, P. R. J. Chromatogr. 1984, 299, 301.

(26) Small, H.; Miller, T. E. Anal. Chem. 1982, 54, 462.

(27) Miller, Mourey, Guistina from the Eastman Kodak Company, Rochester, NY.

(28) Gjerde, D. T.; et al. p. 186, 518.

(29) Willard, H. H.; et al. p. 545.

(30) Small, H.; et al. p. 54.

(31) Small, H.; et al. p. 465.

(32) Weast, R. C.; Astle, M. J.; Eds. "CRC Handbook of Chemistry and Physics A Ready-Reference Book of Chemical and Physical Data", 63rd. ed.; CRC Press, Inc.: Boca Raton, FL, 1983, B-99.

(33) "Kirk-Othmer Encyclopedia of Chemical Technology", 3rd. ed., Vol 16; John Wiley and Sons: New York, NY, 1982, p. 233.

(34) Harris, D. C. "Quantitative Chemical Analysis", W. H. Freeman and Company: San Francisco, CA, 1982, p. 660.

(35) Peters, D. G.; et al. p. 330.

(36) Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry, A Comprehensive Text", 3rd. ed.; Interscience Publishers: New York, NY, 1972, p. 917.

(37) Johnson. E. L.; et al. p. 38.

(38) Johnson, E. L.; et al. p. 34-36.

(39) Pohl, C. A.; et al. p. 449.

(40) Kirk-Othmer, Vol. 17, p. 734.

(41) Kirk-Othmer, Vol. 17, p. 728.

(42) Schmitt, G. L.; Pietrzyk, D. J. Anal.Chem. 1985, 57, 2247.